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Moderators of the Relationship Between Fear Extinction Learning and Posttraumatic Stress Disorder

Daniel Valenty Zuj

BArts (Hons.)

School of Medicine (Psychology)

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The research associated with this thesis abides by the International and Australian codes on human and animal experimentation, the guidelines by the Australian Government's Office of the Gene Technology Regulator and the rulings of the Safety, Ethics and Institutional Biosafety Committees of the University.

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Statement of Co-Authorship

The following people and institutions contributed to the publication of work undertaken as part of this thesis:

Candidate	**Mr. Daniel V. Zuj,** School of Medicine (Psychology), University of Tasmania, Australia.
Author 2	**Prof. Kim L. Felmingham,** School of Psychological Sciences, University of Melbourne, Australia.
Author 3	**Dr. Matthew A. Palmer,** School of Medicine (Psychology), University of Tasmania, Australia.
Author 4	**Dr. Miriam J. J. Lommen,** Department of Psychology, University of Groningen, The Netherlands.
Author 5	**Mr. Chia-Ming K. Hsu,** School of Medicine (Psychology), University of Tasmania, Australia.
Author 6	**Miss. Emma L. Nicholson,** School of Medicine (Psychology), University of Tasmania, Australia.
Author 7	**Miss. Pippa J. Cushing,** School of Medicine (Psychology), University of Tasmania, Australia.
Author 8	**Miss. Kate E. Gray,** School of Medicine (Psychology), University of Tasmania, Australia.
Author 9	**Prof. Gin S. Malhi,** Royal North Shore Hospital, Sydney & Sydney Medical School, University of Sydney, Australia.
Author 10	**Prof. Richard A. Bryant,** School of Psychology, University of New South Wales, Australia.

Author details and their roles:

Paper 1, The centrality of fear extinction in linking risk factors to PTSD: A narrative review

Located in Chapter 2

Candidate was the primary author (70%), and with authors 2 and 3 contributed to the idea, its formalization and development, refinement and presentation (25%). Author 4 contributed to refinement and presentation (5%).

Paper 2, Impaired fear extinction associated with PTSD increases with hours-since-waking

Located in Chapter 3

Candidate was the primary author (65%), and with authors 2 and 3 contributed to the idea, its formalization and development, refinement and presentation (30%). Authors 5, 6, 7 and 8 contributed to participant recruitment and data collection (5%).

Paper 3, Negative appraisals and fear extinction are independently related to PTSD symptoms
Located in Chapter 4
Candidate was the primary author (65%), and with authors 2 and 3 contributed to the idea, its formalization and development, refinement and presentation (25%). Authors 5, 6 and 8 contributed to participant recruitment and data collection (5%). Authors 9 and 10 contributed to refinement and presentation (5%).

Paper 4, Endogenous noradrenaline does not interact with PTSD-related fear extinction learning impairment
Located in Chapter 5
Candidate was the primary author (65%), and with authors 2 and 3 contributed to the idea, its formalization and development, refinement and presentation (30%). Authors 9 and 10 contributed to refinement and presentation (5%).

Paper 5, Endogenous cortisol reactivity moderates the relationship between fear inhibition to safety signals and posttraumatic stress disorder symptoms
Located in Chapter 6
Candidate was the primary author (65%), and with authors 2 and 3 contributed to the idea, its formalization and development, refinement and presentation (30%). Authors 9 and 10 contributed to refinement and presentation (5%).

We the undersigned agree with the above-stated "proportion of work undertaken" for each of the above published (or submitted) peer-reviewed manuscripts contributing to this thesis:

Signed: Candidate _____

Author 2 _____

Author 3 _____

Author 4 _____

Author 5 _____

Author 6 _____

Author 7 _____

Author 8 _____

Author 9 _____

Author 10 _____

Head of School Declaration

I the undersigned agree with the above-stated "proportion of work undertaken" for each of the above published (or submitted) peer-reviewed manuscripts contributing to this thesis:

Signed:

Date: October 11, 2016

Professor Ben Canny
Head of School
School of Medicine
University of Tasmania

Publications

Publications directly arising from the work described in this thesis:

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Zuj, D. V., Palmer, M. A., Hsu, C. K., Nicholson, E. L., Cushing, P. J., Gray, K. E., & Felmingham, K. L. (2016). Impaired fear extinction associated with PTSD increases with hours-since-waking. *Depression and Anxiety, 33*(3), 203-210. doi: 10.1002/da.22463

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Zuj, D. V. (2014). *Can fear of a circle predict long-term fear from a trauma?* 3-Minute Thesis competition finalist at the UTAS Graduate Research Conference, Hobart, Australia.

Zuj, D. V., Palmer, M. A., Vickers, J., & Felmingham, K. L. (2013). *The relationship between REM sleep, verbal function, and fear extinction in posttraumatic stress disorder.* The Tasmanian Health Science HDR Student Conference, Hobart, Australia.

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An unbiased, unwavering, and unforgettable friend.

Abstract

Background: Posttraumatic stress disorder (PTSD) is a psychiatric condition that occurs following a severe traumatic event, and is associated with distressing intrusive memories, avoidance of trauma reminders, hyperarousal, and negative (alterations in) cognitions and mood. A key theoretical model posits that symptoms persist, in part, due to impaired extinction of conditioned fear. Recent evidence suggests that the fear extinction account of PTSD is a complex multifaceted framework of important biological and cognitive features, including sleep quality, catastrophic negative appraisals, noradrenaline activity, and cortisol output. Little research, however, has examined these factors in relation to fear extinction in a PTSD population. Thus, the overall aim of the current thesis was to investigate factors that may moderate the relationship between fear extinction learning and PTSD symptom severity, thereby providing a greater understanding of the variables that shape the role of fear extinction learning in PTSD.

Method: The current thesis includes a narrative review of relevant literature and four empirical studies, each assessing the moderating role of different key variables: (1) hours-since-waking (as a proxy for homeostatic sleep pressure); (2) catastrophic negative appraisals; (3) noradrenaline activity; and (4) cortisol output. These empirical studies used a cross-sectional sample of participants with PTSD, compared to trauma-exposed and non-exposed controls. Participants completed a standardized fear conditioning and extinction paradigm, providing skin conductance response as the primary index of conditioned responding. The PTSD Checklist was used as a diagnostic instrument to ascertain PTSD status and also provided an ordinal measure of PTSD symptom severity.

Results: PTSD was associated with a number of fear extinction learning impairments, including altered responding to the CS+/- during the late extinction phase, and slower extinction learning during the early extinction phase, compared to trauma-exposed and non-exposed controls. Moderation analyses revealed that hours-since-waking and cortisol reactivity were significant moderators between fear extinction learning and PTSD symptom severity. Additionally, catastrophic negative appraisals and fear extinction learning were both associated with PTSD symptom severity, however there was no moderation interaction. Endogenous noradrenaline activity did not moderate the relationship between fear extinction and PTSD symptoms.

Conclusions: The findings presented in the current thesis have a number of implications for the theoretical account of impaired fear extinction learning in PTSD, and for the implementation of exposure-based therapies for PTSD. In particular, we suggest that exposure therapies may be more beneficial if scheduled earlier in the day, rather than later; and recent findings that elevating cortisol leads to greater exposure therapy response in PTSD may be successful via enhancing fear extinction to safety signals. Finally, the results of the current thesis also suggest important biological and cognitive elements in PTSD symptoms, which may benefit from targeted treatment strategies.

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Chapter 1

Introduction

1.1 Posttraumatic Stress Disorder

In the current international climate, traumatic events are plentiful, with each event carrying unique psychological consequences. One particular psychiatric outcome of such events is posttraumatic stress disorder (PTSD). In the United States alone, almost half a million veterans sought treatment for PTSD in 2006, with an estimated cost of 3 billion dollars (Haagen, Smid, Knipscheer, & Kleber, 2015; Institute of Medicine (IOM), 2008). Symptoms of PTSD include distressing intrusive memories, avoidance of trauma reminders, hyperarousal, and negative cognitions and mood (American Psychiatric Association, 2013). PTSD is a unique psychiatric condition, in that we know the specific catalyst for the disorder's etiology: the traumatic event. While the incidence of trauma exposure is quite high, only 10-15% of trauma survivors develop PTSD (Kessler, Sonnega, Bromet, Hughes, & Nelson, 1995; Ramchand et al., 2010). This leads to an important foundational question: why does PTSD develop in some, while others remain resilient?

On the basis of Pavlov's classical conditioning theory (Pavlov, 1927), symptoms of intrusive memories (and to some degree, avoidance behaviors and hyperarousal) have been theorized as conditioned emotional fear responses arising from the trauma (Mineka & Oehlberg, 2008). Indeed, fear conditioning is considered one of the primary explanations for the development of fear-related PTSD symptoms (e.g., Mineka & Oehlberg, 2008; Pitman, 1988; Pitman et al., 2012), and while conditioning can explain the onset of PTSD symptoms, the majority of trauma survivors recover without treatment (Bryant, 2003, 2011). Therefore, other processes must be involved in the clinical persistence of symptoms. A key biological model of PTSD proposes that impaired extinction of such associative fears may explain persistent symptomatology (Pitman et al., 2012). Indeed, prospective studies have identified impaired fear extinction ability as a pre-trauma risk factor for PTSD in firefighters and combat veterans (Guthrie & Bryant, 2006; Lommen, Engelhard, Sijbrandij, van den Hout, &

Hermans, 2013). Further, a recent meta-analysis found that PTSD is consistently associated with slower extinction of conditioned fear to a threat signal, and impaired inhibition of fear to safety signals during the acquisition of fear (Duits et al., 2015). In this meta-analysis, the authors highlight an important need to further investigate fear extinction learning in PTSD, as extinction is considered to underlie treatment success (Duits et al., 2015; Graham & Milad, 2011).

1.2 The Importance of Understanding Fear Extinction: Relation to Treatment

Cognitive behavioral therapy (CBT) for PTSD often involves consciously engaging with traumatic memories with the aim of correcting catastrophic beliefs about the trauma and to reduce the fear response to trauma-related memories. Exposure therapy is the most recommended treatment option for PTSD (Foa, Keane, Friedman, & Cohen, 2009; Karlin et al., 2010; Susskind, Ruzek, & Friedman, 2012; Yehuda et al., 2015). Exposure-based therapies act on the principle of fear extinction by correcting catastrophic emotional responses to memories and reminders of the trauma, as well as reducing avoidance of triggers for trauma-related emotions (Foa & Kozak, 1986). Despite the well-established efficacy of prolonged exposure therapy for PTSD, initial treatment sessions involve recounting the trauma memory, which may result in severe symptom expression (Yehuda et al., 2015). Unfortunately, the severity of symptom expression in early stages of treatment can lead to high rates of treatment drop-out (Schnurr et al., 2007; Simmons, Norman, Spadoni, & Strigo, 2013; Yehuda et al., 2015). With impaired fear extinction forming the theoretical foundation of PTSD symptom persistence and treatment, it is important to investigate the factors that may shape the relationship between impaired fear extinction and PTSD symptomatology. The current thesis presents a series of investigations into the role of

fear extinction learning in PTSD, and additional factors that may moderate the relationship between fear extinction and PTSD.

1.3 Aim of the Present Thesis and Outline of Chapters

Despite the proven efficacy of prolonged exposure therapy as a treatment option for PTSD (Yehuda et al., 2015), approximately 30% of patients only display partial recovery (Foa et al., 1999; Foa & McLean, 2016). As fear extinction is the underlying principle and goal of exposure-based therapies, factors known to aid or impair extinction ability may have important implications for maximizing treatment response (Graham, Callaghan, & Richardson, 2014). As such, the overall aim of the present thesis was to investigate a number of factors that may moderate the relationship between fear extinction learning and PTSD symptoms, in an experimental framework.

Chapter two presents a comprehensive narrative review of the relationship between key biological and cognitive risk factors for PTSD, and their associations with impaired fear extinction learning and memory. Specifically, this narrative review discusses the central role that impaired fear extinction appears to play in linking risk factors to PTSD. Due to the extensive nature of this review and the relevance for the subsequent empirical chapters, this peer-reviewed article has been included as the primary literature review for the current thesis. Chapter two as presented has been published:

Zuj, D. V., Palmer, M. A., Lommen, M. J. J., & Felmingham, K. L. (2016). The centrality of fear extinction in linking risk factors to PTSD: A narrative review. *Neuroscience and Biobehavioral Reviews, 69,* 15-35. doi:10.1016/j.neubiorev.2016.07.014.

In the narrative review article presented in Chapter two, a number of key variables are discussed that share connections with impaired fear extinction and PTSD. The

subsequent chapters detail investigations into a select few of these variables, to examine any interactions between these factors and impaired fear extinction in participants with current PTSD, compared to trauma-exposed and non-trauma-exposed controls. Regarding Chapter three, previous research in healthy males suggests that time-of-day plays an important role in the extinction of conditioned fear, with extinction better learned and generalized in the morning compared to the evening (Pace-Schott et al., 2013). This finding suggests that time-of-day may moderate fear extinction, with extinction worsening over the course of the day. Further, this effect has not been investigated in PTSD. Chapter three addresses this question by hypothesizing that PTSD would be associated with impaired fear extinction learning, and that this relationship would become stronger with increased hours-since-waking. Chapter three as presented has been published:

Zuj, D. V., Palmer, M. A., Hsu, C. K., Nicholson, E. L., Cushing, P. J., Gray, K. E., & Felmingham, K. L. (2016). Impaired fear extinction associated with PTSD increases with hours-since-waking. *Depression and Anxiety, 33*, 203-210. doi:10.1002/da.22463.

The cognitive theory of PTSD (Ehlers & Clark, 2000) proposes that negative appraisals of the trauma and its sequelae are key features of persistent PTSD symptoms. Further, intrusive memories and conditioned fear responses triggered by trauma reminders are thought to reinforce negative appraisals, thereby maintaining anxiety levels and a sense of current threat. Longitudinal evidence has found that PTSD symptoms can be predicted by poor pre-trauma fear extinction learning (Guthrie & Bryant, 2006), and maladaptive pre-trauma negative appraisals (Bryant & Guthrie, 2007). Therefore, impaired extinction capacity pre-trauma and heightened negative appraisals may interact to potentiate fear

responses following trauma. Chapter four investigates the possible interaction between fear extinction learning and negative appraisals in PTSD. Specifically, negative appraisals are assessed as a moderator between fear extinction and PTSD. In doing so, this study attempts to integrate two prevailing models of PTSD: the cognitive theory of PTSD (Ehlers & Clark, 2000), and the biological fear extinction account of PTSD (e.g., Mineka & Oehlberg, 2008; Pitman et al., 2012). This study is currently under peer-review:

Zuj, D. V., Palmer, M. A., Gray, K. E., Hsu, C. K., Malhi, G. S., Bryant, R. A., & Felmingham, K. L. Negative appraisals and fear extinction are independently related to PTSD symptoms. *Journal of Affective Disorders [JAD_2016_1657]*.

PTSD is a disorder that includes heightened arousal and fear, and as such, certain stress hormones have been implicated in PTSD and fear extinction. The stress response typically involves two waves of neurochemical release. The first wave involves increased noradrenergic signaling for the purpose of activating sympathetic arousal structures to engage with the stressor (Antov, Melicherová, & Stockhorst, 2015). The hypothalamic-pituitary-adrenal (HPA) axis is the premier neuroendocrine system in the mammalian brain, and its end-product, cortisol, is involved in the second wave of the stress response, aimed at returning sympathetic arousal structures to homeostasis (Joëls & Baram, 2009). Chapter five examines the role of endogenous salivary noradrenaline in moderating the relationship between fear extinction learning and PTSD, while Chapter six examines endogenous salivary cortisol as a moderator.

PTSD is consistently associated with hyperactive noradrenergic signaling (for a review see Zoladz & Diamond, 2013), and behavioral studies in healthy controls have revealed that increased noradrenaline release prior to fear acquisition results in a conditioned

fear memory that is resistant to extinction (Antov, Wolk, & Stockhorst, 2013). This finding suggests that the relationship between impaired fear extinction and PTSD symptoms may become stronger as basal noradrenaline levels increase. Chapter five addresses this question, and is currently in preparation for submission for peer-review:

Zuj, D. V., Palmer, M. A., Malhi, G. S., Bryant, R. A., & Felmingham, K. L. Endogenous noradrenaline does not interact with PTSD-related fear extinction learning impairments.

Regarding the second wave of the stress response, a recent meta-analysis found consistent evidence of low cortisol output in PTSD, after taking methodological variations into account (Morris, Compas, & Garber, 2012). Further, studies show that PTSD patients demonstrate enhanced suppression of cortisol in response to dexamethasone administration, which is suggested as evidence of low cortisol activity and hypersensitive glucocorticoid receptors (Yehuda et al., 1993). Chapter six presents an investigation into the role of cortisol activity in the relationship between fear extinction learning and PTSD symptoms. Based on previous research, we hypothesized that better fear extinction learning ability would be associated with lower PTSD symptoms, and that this relationship would be stronger for participants with greater cortisol output. Chapter six as presented has been published:

Zuj, D. V., Palmer, M. A., Malhi, G. S., Bryant, R. A., & Felmingham, K. L. (2017). Endogenous cortisol reactivity moderates the relationship between fear inhibition to safety signals and posttraumatic stress disorder symptoms. *Psychoneuroendocrinology, 78*, 14-21. doi:10.1016/j.psyneuen.2017.01.012

Chapter seven concludes with a general discussion that summarizes the key findings, implications, and limitations of the current thesis. Directions for future research in fear extinction and PTSD are also discussed.

1.4 References

American Psychiatric Association. (2013). *Diagnostic and Statistical Manual of Mental Disorders* (5th ed.). Washington, DC: Author.

Antov, M. I., Melicherová, U., & Stockhorst, U. (2015). Cold pressor test improves fear extinction in healthy men. *Psychoneuroendocrinology, 54*, 54-59. doi:10.1016/j.psyneuen.2015.01.009

Antov, M. I., Wolk, C., & Stockhorst, U. (2013). Differential impact of the first and second wave of a stress response on subsequent fear conditioning in healthy men. *Biological Psychology, 94*(2), 456-468. doi:10.1016/j.biopsycho.2013.08.007

Bryant, R. A. (2003). Early predictors of posttraumatic stress disorder. *Biological Psychiatry, 53*(9), 789-795. doi:10.1016/S0006-3223(02)01895-4

Bryant, R. A. (2011). Acute stress disorder as a predictor of posttraumatic stress disorder: A systematic review. *Journal of Clinical Psychiatry, 72*(2), 233-239. doi:10.4088/JCP.09r05072blu

Bryant, R. A., & Guthrie, R. M. (2007). Maladaptive self-appraisals before trauma exposure predict posttraumatic stress disorder. *Journal of Consulting and Clinical Psychology, 75*(5), 812-815. doi:10.1037/0022-006x.75.5.812

Duits, P., Cath, D. C., Lissek, S., Hox, J. J., Hamm, A. O., Engelhard, I. M., . . . Baas, J. M. P. (2015). Updated meta-analysis of classical fear conditioning in the anxiety disorders. *Depression and Anxiety, 32*(4), 239-253. doi:10.1002/da.22353

Ehlers, A., & Clark, D. M. (2000). A cognitive model of posttraumatic stress disorder. *Behaviour Research and Therapy, 38*, 319-345.

Foa, E. B., & Kozak, M. J. (1986). Emotional processing of fear: exposure to corrective information. *Psychological Bulletin, 99*(1), 20-35.

Foa, E. B., Dancu, C. V., Hembree, E. A., Jaycox, L. H., Meadows, E. A., & Street, G. P. (1999). A comparison of exposure therapy, stress inoculation training, and their combination for reducing posttraumatic stress disorder in female assault victims. *Journal of Consulting and Clinical Psychology, 67*(2), 194-200.

Foa, E. B., Keane, T. M., Friedman, M. J., & Cohen, J. A. (Eds.). (2009). *Effective treatments for PTSD: Practice guidelines from the International Society of Traumatic Stress Studies* (2nd ed.). New York: Guilford.

Foa, E. B., & McLean, C. P. (2016). The Efficacy of Exposure Therapy for Anxiety-Related Disorders and Its Underlying Mechanisms: The Case of OCD and PTSD. *Annual Review of Clinical Psychology, 12*, 1-28. doi:10.1146/annurev-clinpsy-021815-093533

Graham, B. M., Callaghan, B. L., & Richardson, R. (2014). Bridging the gap: Lessons we have learnt from the merging of psychology and psychiatry for the optimisation of treatments for emotional disorders. *Behaviour Research and Therapy, 62*, 3-16. doi:10.1016/j.brat.2014.07.012

Graham, B. M., & Milad, M. R. (2011). The study of fear extinction: Implications for anxiety disorders. *The American Journal of Psychiatry, 168*(12), 1255-1265. doi:10.1176/appi.ajp.2011.11040557

Guthrie, R. M., & Bryant, R. A. (2006). Extinction learning before trauma and subsequent posttraumatic stress. *Psychosomatic Medicine, 68*, 307-311. doi:10.1097/01.psy.0000208629.67653.cc

Haagen, J. F. G., Smid, G. E., Knipscheer, J. W., & Kleber, R. J. (2015). The efficacy of recommended treatments for veterans with PTSD: A metaregression analysis. *Clinical Psychology Review, 40*, 184-194. doi: 10.1016/j.cpr.2015.06.008

Institute of Medicine (IOM). (2008). *Treatment of posttraumatic stress disoder: An assessment of the evidence*. Washington DC: The National Academies Press.

Joëls, M., & Baram, T. Z. (2009). The neuro-symphony of stress. *Nature Reviews Neuroscience, 10*(6), 459-466. doi:10.1038/nrn2632

Karlin, B. E., Ruzek, J. I., Chard, K. M., Eftekhari, A., Monson, C. M., Hembree, E. A., . . . Foa, E. B. (2010). Dissemination of evidence-based psychological treatments for posttraumatic stress disorder in the Veterans Health Administration. *Journal of Traumatic Stress, 23*(6), 663-673. doi:10.1002/jts.20588

Kessler, R. C., Sonnega, A., Bromet, E., Hughes, M., & Nelson, C. B. (1995). Posttraumatic stress disorder in the National Comorbidity Survey. *Archives of General Psychiatry, 52*(12), 1048-1060.

Lommen, M. J. J., Engelhard, I. M., Sijbrandij, M., van den Hout, M. A., & Hermans, D. (2013). Pre-trauma individual differences in extinction learning predict posttraumatic stress. *Behaviour Research and Therapy, 51*(2), 63-67. doi:10.1016/j.brat.2012.11.004

Mineka, S., & Oehlberg, K. (2008). The relevance of recent developments in classical conditioning to understanding the etiology and maintenance of anxiety disorders. *Acta Psychologica, 127*(3), 567-580. doi:10.1016/j.actpsy.2007.11.007

Morris, M. C., Compas, B. E., & Garber, J. (2012). Relations among posttraumatic stress disorder, comorbid major depression, and HPA function: a systematic review and meta-analysis. *Clinical Psychology Review, 32*(4), 301-315. doi:10.1016/j.cpr.2012.02.002

Pace-Schott, E. F., Spencer, R. M. C., Vijayakumar, S., Ahmed, N. A. K., Verga, P. W., Orr, S. P., . . . Milad, M. R. (2013). Extinction of conditioned fear is better learned and recalled in the morning than in the evening. *Journal of Psychiatric Research, 47*(11), 1776-1784. doi:10.1016/j.jpsychires.2013.07.027

Pavlov, I. P. (1927). *Conditioned reflexes: An investigation of the physiological activity of the cerebral cortex*. London: Oxford University Press.

Pitman, R. K. (1988). Post-traumatic stress disorder, conditioning, and network theory. *Psychiatric Annals, 18*(3), 182-&.

Pitman, R. K., Rasmusson, A. M., Koenen, K. C., Shin, L. M., Orr, S. P., Gilbertson, M. W., . . . Liberzon, I. (2012). Biological studies of post-traumatic stress disorder. *Nature, 13*, 769-787. doi:10.1038/nm3339

Ramchand, R., Schell, T. L., Karney, B. R., Osilla, K. C., Burns, R. M., & Caldarone, L. B. (2010). Disparate prevalence estimates of PTSD among service members who served in Iraq and Afghanistan: possible explanations. *Journal of Traumatic Stress, 23*(1), 59-68. doi:10.1002/jts.20486

Schnurr, P. P., Friedman, M. J., Engel, C. C., Foa, E. B., Shea, M. T., Chow, B. K., . . . Bernardy, N. (2007). Cognitive behavioral therapy for posttraumatic stress disorder in women: a randomized controlled trial. *Journal of the American Medical Association, 297*(8), 820-830. doi:10.1001/jama.297.8.820

Simmons, A. N., Norman, S. B., Spadoni, A. D., & Strigo, I. A. (2013). Neurosubstrates of remission following prolonged exposure therapy in veterans with posttraumatic stress disorder. *Psychotherapy and Psychosomatics, 82*(6), 382-389. doi:10.1159/000348867

Susskind, O., Ruzek, J. I., & Friedman, M. J. (2012). The VA/DOD clinical practice guideline for management of post-traumatic stress (update 2010): Development and methodology. *Journal of Rehabilitation Research and Development, 49*(5), xvii-xxviii.

Yehuda, R., Bierer, L. M., Pratchett, L. C., Lehrner, A., Koch, E. C., Van Manen, J. A., . . . Hildebrandt, T. (2015). Cortisol augmentation of a psychological treatment for warfighters with posttraumatic stress disorder: Randomized trial showing improved treatment retention and outcome. *Psychoneuroendocrinology, 51*, 589-597. doi:10.1016/j.psyneuen.2014.08.004

Yehuda, R., Southwick, S. M., Krystal, J. H., Bremner, D., Charney, D. S., & Mason, J. W. (1993). Enhanced suppression of cortisol following dexamethasone administration in posttraumatic stress disorder. *The American Journal of Psychiatry, 150*(1), 83-86. doi:10.1176/ajp.150.1.83

Zoladz, P. R., & Diamond, D. M. (2013). Current status on behavioral and biological markers of PTSD: A search for clarity in a conflicting literature. *Neuroscience and Biobehavioral Reviews, 37*(5), 860-895. doi:10.1016/j.neubiorev.2013.03.024

Chapter 2

The Centrality of Fear Extinction in Linking Risk Factors to PTSD: A Narrative Review

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2.1 Abstract

Recent prospective studies in emergency services have identified impaired fear extinction learning and memory to be a significant predictor of posttraumatic stress disorder (PTSD), complementing a wealth of cross-sectional evidence of extinction deficits associated with the disorder. Additional fields of research show specific risk factors and biomarkers of the disorder, including candidate genotypes, stress and sex hormones, cognitive factors, and sleep disturbances. Studies in mostly nonclinical populations also reveal that the aforementioned factors are involved in fear extinction learning and memory. Here, we provide a comprehensive narrative review of the literature linking PTSD to these risk factors, and linking these risk factors to impaired fear extinction. On balance, the evidence suggests that fear extinction may play a role in the relationship between risk factors and PTSD. Should this notion hold true, this review carries important implications for the improvement of exposure-based treatments, as well as strategies for the implementation of treatment.

2.2 Introduction

Epidemiological studies indicate that over half of the population in the United States will be exposed to a serious traumatic event in their lifetime (Kessler, Sonnega, Bromet, Hughes, & Nelson, 1995). One particular psychiatric outcome of trauma exposure is posttraumatic stress disorder (PTSD), characterized by distressing re-experiencing, avoidance, negative (alterations in) cognitions and mood, and hyperarousal symptoms (American Psychiatric Association, 2013). Further, PTSD is associated with considerable psychological distress, elevated risk of suicide, and comorbid psychiatric disorders. Despite high rates of trauma exposure, only 10-15% of individuals develop PTSD (Kessler et al., 1995; Ramchand et al., 2010), constituting approximately 3% of the population in the United States (Kessler, Chiu, Demler, Merikangas, & Walters, 2005). PTSD is unique among psychiatric disorders, as we know the triggering event; however it is unclear why the disorder develops in some but not in others. Research indicates that the majority of trauma survivors show severe symptoms of acute stress disorder (ASD) and PTSD in the weeks following trauma, however these rates drop dramatically in the following months (for a review, see Bryant, 2003). Therefore, the normative response to trauma is recovery. A significant body of scientific research has uncovered important neurobiological, cognitive, and psychological correlates of the disorder. A compelling and unresolved issue is *what discriminates those who develop PTSD from those who experience trauma but remain resilient?* This highlights the importance of identifying key risk and resilience factors, biomarkers, and underlying mechanisms that may mediate/moderate the relationship between a traumatic event and PTSD symptoms.

A prevailing model suggests that the impaired extinction of conditioned fear in the aftermath of trauma is critical in the development and maintenance of PTSD (e.g., Mineka & Oehlberg, 2008; Pitman et al., 2012). There is substantial evidence of greater fear

conditioning and impaired fear extinction in patients with PTSD (Blechert, Michael, Vriends, Margraf, & Wilhelm, 2007; Norrholm et al., 2011; Zuj et al., 2016). However, to examine the role of fear extinction in the development of PTSD, longitudinal prospective studies are required that examine these processes prior to and following trauma exposure. Recent prospective studies have revealed that impaired fear extinction prior to trauma exposure predicts increased PTSD symptom severity (Guthrie & Bryant, 2006; Lommen, Engelhard, Sijbrandij, van den Hout, & Hermans, 2013; Orr et al., 2012). The use of prospective studies has allowed the identification of other key risk factors identified in PTSD development, including candidate genotypes (Matsuoka, Nishi, Noguchi, Kim, & Hashimoto, 2013), stress and sex hormones (Bryant et al., 2011; Videlock et al., 2008), cognitive factors (Vasterling et al., 2012), and sleep disturbances (Bryant, Creamer, O'Donnell, Silove, & McFarlane, 2010). Interestingly, the connection between these risk factors and impaired extinction learning in PTSD has not received much attention yet. To date, certain links have been identified between fear extinction and candidate genotypes (Johnson, McGuire, Lazarus, & Palmer, 2012), stress and sex hormones (Milad et al., 2010; Mueller & Cahill, 2010), cognitive factors (Raes, De Raedt, Verschuere, & De Houwer, 2009), and sleep disturbances (Spoormaker et al., 2010). On balance, the evidence points to impaired fear extinction as a shared factor that may link these biomarkers and risk factors to PTSD, although we acknowledge certain inconsistencies in the literature. In this review, we outline the evidence that suggests a fundamental role of fear extinction, and review an important question: is fear extinction the central factor that links known risk factors to PTSD?

2.2.1 Focus of this review.

Impaired fear extinction learning and memory appear to be key variables that link risk factors to PTSD susceptibility, and the aim of this review is to highlight these convergences. This paper will address this issue by providing a comprehensive review of the pre-trauma risk and resilience factors of PTSD development, and the evidence linking these factors to impaired fear extinction processes. Classic PTSD signs and symptoms include fear and anxiety, catastrophic cognitions, anger, substance abuse, shame, and guilt. It is important to note that this review focuses on the fear-related symptoms of PTSD (e.g., intrusive memories and re-experiencing symptoms), which are often considered primary symptoms. First, an overview of the current fear conditioning and extinction model of PTSD will be provided, including a brief review of convergent animal and human research on the neural networks of fear extinction relating to PTSD, and a summary of emerging prospective studies in fear extinction and PTSD. Second, prospective and cross-sectional studies examining risk and resilience factors in PTSD across varying domains of genetics, stress and sex hormones, cognitive function, and sleep will be reviewed. Third, we highlight the converging literature bases of known risk factors and impaired fear extinction learning and/or its retention. Based on this, we propose a model in which extinction is the central variable linking risk factors to PTSD, and discuss the current empirical evidence for this model. Finally, we discuss considerations for future research to investigate this model. Should further investigations into the role of fear extinction as a mechanism between risk factors and PTSD hold true, these findings would carry important implications for further research and clinical practice. These implications include advances for theory building and the development of interventions aimed at boosting PTSD resilience in susceptible populations (e.g., emergency services and military personnel).

2.3 The Fear Conditioning and Extinction Model of PTSD

The fear conditioning model is central in explaining the development of PTSD. This model is based on Pavlov's (1927) seminal work in classical conditioning, where a learned association between a previously neutral stimulus (e.g., a light, termed the conditioned stimulus; CS) and an aversive stimulus (unconditioned stimulus; US), leads to heightened US-expectancy of the CS. After learning, the CS alone generates a conditioned fear response in absence of the US. This principle operates when people experience trauma events. For example, an individual involved in a motor vehicle accident (US) is likely to experience a natural response of fear and heightened arousal (the unconditioned response; UCR). Following the event, aspects of the trauma that were not associated with the accident before (e.g., the car), may act as a trigger to activate the trauma memory, eliciting the CR.

While fear conditioning processes can explain the *acquisition* of conditioned fear, most traumatized individuals recover and do not go on to develop PTSD (reviewed in Bryant, 2003). Indeed, the majority of individuals report ASD/PTSD symptoms within the initial weeks following trauma in the form of acute stress disorder (Bryant, 2011), however only a small percentage of these do not recover, and go on to display persistent symptoms. This rapid reduction in PTSD symptoms may be attributed partially to fear extinction learning, which has led current models to emphasize fear extinction processes in the maintenance of PTSD (e.g., Mineka & Oehlberg, 2008; Pitman et al., 2012; VanElzakker, Kathryn Dahlgren, Caroline Davis, Dubois, & Shin, 2013). Fear extinction refers to a process of new learning in which repeated exposure to a CS in the absence of an aversive consequence (the US) leads to a reduction in conditioned fear responses. In the context of trauma, this typically involves experience of benign trauma reminders. In this sense, the minority of trauma survivors who experience persistent conditioned emotional responses and chronic PTSD can be regarded as suffering impaired fear extinction (Davis & Myers,

2002; Kolb & Mutalipassi, 1982). Therefore, a prevailing model of PTSD is that a central component of the disorder results from impaired fear extinction (Mineka & Oehlberg, 2008; Pitman et al., 2012; Shin & Liberzon, 2010).

2.4 Empirical Evidence for Impaired Fear Extinction in PTSD

Recently, research indicates that the persistence of PTSD symptoms nine months post-deployment in Dutch soldiers can be predicted by an impaired ability to inhibit learned fear, as indexed by an increased startle response to a CS in the presence of a safety signal (Sijbrandij, Engelhard, Lommen, Leer, & Baas, 2013). Compared to trauma-exposed controls without PTSD and healthy controls, participants with PTSD demonstrate a robust psychophysiological increase in arousal to the CS+ during the extinction phase, indicative of a reduced ability to extinguish conditioned fear (Blechert et al., 2007; Norrholm et al., 2011; Orr et al., 2000; Peri, Ben-Shakhar, Orr, & Shalev, 2000; Zuj et al., 2016). Further, participants with PTSD have also shown an impaired ability to inhibit conditioned fear responses to a CS that has not been reinforced by the US (Jovanovic et al., 2010; Jovanovic et al., 2009). These findings suggest that PTSD symptoms are associated with impairment in regulating fear responses previously associated with an aversive stimulus, as well as responses to safety signals.

Participants often display over-expressed conditioned fear during the early stage of extinction learning, termed fear load (Norrholm et al., 2015). Fear load, as measured by fear-potentiated startle, has been observed in PTSD (Norrholm et al., 2011), and later identified to be significantly correlated with intrusive fear memories of a traumatic event (Norrholm et al., 2015). Importantly, the authors argue that fear load may be a quantifiable intermediate phenotype with a significant role in the etiology of fear-related psychopathology (Briscione, Jovanovic, & Norrholm, 2014; Norrholm et al., 2015).

Some studies have demonstrated little difference in within-session extinction learning between PTSD subjects and controls (Milad et al., 2008; Milad, Pitman, et al., 2009) but rather found poor recall of extinction memories in PTSD 24 hours after extinction learning (Milad et al., 2008; Milad, Pitman, et al., 2009; Shvil et al., 2014). Shvil et al. (2014) used a two-day paradigm to assess fear conditioning and extinction learning on day one, and fear extinction recall on day two. The results showed impaired recall of fear extinction learning in PTSD, however this result was sex-specific, with men showing poorer extinction recall compared to women with PTSD (Shvil et al., 2014). Additional studies using a similar two-day paradigm have found further support for impaired fear extinction recall in PTSD, albeit without sex differences (Milad et al., 2008; Milad, Pitman, et al., 2009). Together, these findings indicate that impairments may not be limited to extinction learning, but rather to the consolidation of extinction memories, or availability for recall. It is worth noting here, however, that there are certain methodological differences in the fear conditioning and extinction paradigms discussed here, compared to studies discussed in above paragraphs. For example, there are specific differences between paradigms regarding the use of partial or full CS-US reinforcement schedules, the addition of contextual contingencies, pharmacological challenge interventions, and the measurement of conditioned responses via skin conductance response (SCR), fear-potentiated startle, and self-report US-expectancy ratings (e.g., Milad et al., 2008; Norrholm et al., 2011; Soeter & Kindt, 2012). It is possible that these differences in experimental paradigms may account for alternate findings.

The continued expression of fear or spontaneous return of fear following extinction learning suggests that extinction is formed as a new memory that competes with the original conditioning memory, rather than overriding the conditioning trace (for a brief review, see Pace-Schott, Germain, & Milad, 2015a). However, it remains unclear whether PTSD is

associated with impairments in the learning, consolidation, or retrieval of extinction memories, or a combination of all of these processes. Nevertheless, evidence is clear that PTSD is associated with impaired mechanisms of fear extinction.

2.5 The Neurobiology of Fear Conditioning and Extinction

Research in neuroimaging of rodents and humans has led to considerable advances in our understanding of the neural pathways involved in fear expression. This section will provide a brief discussion of the evidence from animal studies, followed by translational evidence in human neuroimaging studies (for a comprehensive review, see Pitman et al., 2012). Finally, this section will present convergent evidence for the role of this neural network in human neuroimaging studies using fear conditioning and extinction paradigms in PTSD subjects.

2.5.1 Animal studies.

The amygdala has been referred to as the neural 'hub of fear' (Milad & Quirk, 2012), with different regions of the amygdala playing key roles in the acquisition of fear. Specifically, the basolateral amygdala (BLA) receives sensory information on the CS-US association, as well as the presence of contextual contingencies, and the central nucleus (CE) sends afferent projections to behavioral and endocrine systems to express fear (Holmes & Singewald, 2013; LeDoux, 2000; Pape & Pare, 2010; Pare & Duvarci, 2012; Pare, Quirk, & Ledoux, 2004). For example, the CE projects to the periaqueductal grey to regulate freezing behaviour in rodents and the lateral hypothalamus to regulate blood pressure (Medina, Repa, Mauk, & LeDoux, 2002). Evidence also links the prelimbic system of the medial prefrontal cortex (mPFC) to the expression of conditioned fear. That is, freezing behavior in rodents is correlated with neuronal activity in the prelimbic cortex (Burgos-Robles, Vidal-Gonzalez, & Quirk, 2009). Similarly, inactivation of the prelimbic cortex is associated with reduced

expression of conditioned fear (Corcoran & Quirk, 2007), supporting the notion that the amygdala receives excitatory projections from the prelimbic cortex to express fear. Emerging evidence also suggests that activation of the basomedial amygdala (BMA) in mice plays a significant role in the reduction of freezing and anxiety behaviors (Adhikari et al., 2015). Furthermore, the ventral mPFC exerts top-down control over the BMA to regulate this process.

The amygdala sends afferent projections to sympathetic arousal centres to express fear, however the mPFC and the hippocampus play a larger role in mediating this process as the CS ceases to predict the US during fear extinction. Greater activation of the infralimbic cortex is associated with enhanced extinction recall (Milad & Quirk, 2002) and electrical stimulation of infralimbic neurons resulted in reduced conditioned fear responses (i.e., reduced freezing in rodents; Milad, Vidal-Gonzalez, & Quirk, 2004). In further support, lesions to the infralimbic cortex are associated with increased extinction recall (Laurent & Westbrook, 2009; Quirk, Russo, Barron, & Lebron, 2000). Presentation of a CS in an extinguished context has been associated with reduced conditioned freezing and enhanced cFos expression (an indicator of increased neuronal activity) in the infralimbic cortex (Hefner et al., 2008; Knapska & Maren, 2009). Alternatively, presentation of the CS in a non-extinguished context however, has resulted in high levels of conditioned freezing and greater cFos activity in the prelimbic cortex (Knapska & Maren, 2009). These findings suggest that the infralimbic cortex acts in opposition to the prelimbic cortex, with greater activity inhibiting the amygdala, and thereby inhibiting fear expression.

The hippocampus has been implicated in the contextual modulation of extinction learning. Corcoran and Maren (2001) found that when hippocampal activity went uninterrupted, rats showed context-specific freezing behavior. Fear expression was reduced in the extinction context, but elevated in non-extinguished contexts, supporting the notion

that extinction learning is context specific. In a separate experiment, inactivation of the dorsal hippocampus resulted in reduced freezing to the CS when placed in either the extinguished or unextinguished context (Corcoran & Maren, 2001), indicating a role for the dorsal hippocampus in identifying safe versus threatening contexts. It is argued that the hippocampus projects directly to the BLA and indirect connections via the mPFC to relay contextual contingencies on the CS-US relationship (Orsini, Kim, Knapska, & Maren, 2011; Orsini & Maren, 2012). Therefore, when the CS no longer predicts the US, the hippocampus and infralimbic cortex send inhibitory projections to the amygdala, reducing the expression of conditioned fear and demonstrating successful fear extinction retention (for a comprehensive review on the hippocampus and contextual fear conditioning and extinction, see Maren, Phan, & Liberzon, 2013).

2.5.2 Human studies.

A remarkable aspect of fear conditioning and extinction is the potential for translational work from rodents to humans, with the goal of improving clinical interventions and treatment (Milad & Quirk, 2012; Quirk & Mueller, 2008; Rothbaum & Davis, 2003). Activation of the amygdala is enhanced during the acquisition of fear (Cheng, Knight, Smith, Stein, & Helmstetter, 2003; Cheng, Richards, & Helmstetter, 2007; LaBar, Gatenby, Gore, LeDoux, & Phelps, 1998; Morris & Dolan, 2004). Consistent with animal studies, the BLA receives sensory information on the CS-US contingency during fear conditioning and projects this information to sympathetic arousal structures (for a comprehensive review, see LeDoux, 2000). Neuroimaging studies have found the dACC in humans to be the functional homologue of the prelimbic system in rodents (VanElzakker et al., 2013). Cortical thickness and activation of the dACC is positively correlated with SCR during the acquisition of fear, as well as presentations of the CS+ (Milad, Quirk, et al., 2007). Further studies have also

found increased dACC activation during fear conditioning (Buchel, Morris, Dolan, & Friston, 1998; Cheng et al., 2003; Knight, Smith, Cheng, Stein, & Helmstetter, 2004; Linnman, Rougemont-Bucking, Beucke, Zeffiro, & Milad, 2011; Phelps, Delgado, Nearing, & LeDoux, 2004).

The ventromedial PFC (vmPFC) is suggested to be homologous to the rodent infralimbic cortex (Milad, Rauch, Pitman, & Quirk, 2006; Milad, Wright, et al., 2007). Showing parallels with the proposition that the infralimbic cortex may inhibit the amygdala in rodents (e.g., Milad & Quirk, 2002), Motzkin, Philippi, Wolf, Baskaya, and Koenigs (2014) recently showed that vmPFC lesions in human subjects prevented the inhibition of amygdala activity in viewing aversive images, resulting in greater amygdala activity. Similarly, Milad, Wright, et al. (2007) found that the recall of extinction learning was positively correlated with activation of the vmPFC. Milad, Wright, et al. (2007) used an ABB conditioning and extinction design, whereby fear was acquired in context A, but extinguished in context B, with extinction recall also occurring in context B the next day. Extinction recall on day two was associated with increased hippocampal activation, suggesting an important role of the hippocampus in differentiating the acquisition versus extinction contexts (Milad, Wright, et al., 2007). Previous fMRI research using only one context for stimulus presentations found no evidence of hippocampal activation in association with vmPFC activation during extinction recall (Phelps et al., 2004). Milad, Wright, et al. (2007) speculate that this difference is due to contextual signaling in the hippocampus, with this signaling only occurring in instances of multiple contexts with different extinction contingencies. Indeed, Lang et al. (2009) identified increased hippocampal activation where multiple contextual conditioning/extinction contingencies were employed, supporting previous animal studies (Corcoran & Maren, 2001).

2.6 Neuroimaging of Fear Conditioning and Extinction in PTSD

Neuroimaging studies in clinical PTSD samples provide additional support for the role of aforementioned neural structures in the acquisition and extinction of conditioned fear. During fear acquisition, participants with PTSD show significantly greater amygdala activation compared to trauma-exposed controls (Linnman, Zeffiro, Pitman, & Milad, 2011; Milad, Pitman, et al., 2009) and trauma non-exposed controls (Bremner et al., 2005). Although increased amygdala activation is found during fear acquisition in healthy subjects (Cheng et al., 2003; Cheng et al., 2007; LaBar et al., 1998; Morris & Dolan, 2004), it is possible the amygdala is hyperactive in PTSD. In support, increased avoidance symptoms correlate with increased amygdala, vmPFC, and hippocampus activity during both fear acquisition and extinction in United States military veterans with PTSD (Sripada, Garfinkel, & Liberzon, 2013).

PTSD is associated with hyperactivity in the dACC during fear acquisition and extinction learning (Rougemont-Bücking et al., 2011), and extinction recall 24 hours later (Milad, Pitman, et al., 2009; Rougemont-Bücking et al., 2011). Rougemont-Bücking et al. (2011) used a two-day paradigm measuring fear conditioning, extinction (day one), and extinction recall (day two) with fMRI scanning in PTSD subjects. PTSD subjects and healthy trauma-exposed controls acquired fear conditioned responses in context A, with extinction learning and recall occurring in context B. PTSD subjects showed greater dACC activation during conditioning, and reduced vmPFC activity during late extinction learning. A specific limitation of these findings, however, is that there were no between-group differences in SCR (a measure of conditioned responses) at any stage of conditioning or extinction. Although it is, therefore, difficult to attribute activation in these neural regions to greater conditioned responses, neural activity does support the proposed function of these regions at different stages of conditioning and extinction (Milad, Quirk, et al., 2007; Milad,

Wright, et al., 2007). Further, Shvil et al. (2014) recently found that greater left rostral dACC activity was associated with poor fear extinction recall in men with PTSD, with no effects in women with PTSD and trauma-exposed healthy controls.

Milad, Pitman, et al. (2009) used a two-day paradigm (explained above, see Rougemont-Bücking et al., 2011) with fMRI scanning in PTSD subjects and trauma-exposed controls. PTSD was associated with greater amygdala activity during fear acquisition on day one, and decreased hippocampal and vmPFC activity during fear extinction recall on day two. Furthermore, extinction recall was associated with increased dACC activity in PTSD subjects, compared to trauma-exposed controls (Milad, Pitman, et al., 2009). Importantly, these findings support previous research of hippocampal involvement when contextual contingencies are involved. That is, PTSD subjects showed lower hippocampal activation during recall of extinction memories in the extinction context, rather than the conditioning context (Milad, Pitman, et al., 2009). In support, PTSD subjects show lower activity in the parahippocampal gyrus during extinction learning in a different context to an acquisition context (Rougemont-Bücking et al., 2011). Furthermore, a recent fMRI study found increased hippocampal activity during context presentations in extinction learning (Sripada et al., 2013). Alternatively, PET scans during fear conditioning and extinction have revealed increased blood flow in the left hippocampus during extinction learning in PTSD subjects compared to controls, although context was not manipulated in this study (Bremner et al., 2005). These findings implicate a role for the hippocampus in contextual contingencies in fear extinction learning and recall, however further research is required to shed light on whether this structure is only involved in contextual modulation, or other processes of conditioning and extinction.

2.7 Fear Extinction Learning in Twin Studies of PTSD

An important question regarding the neurobiology of PTSD is whether impaired neural signaling is a consequence of a traumatic event, or a biological risk factor that predisposes an individual to overly conditioned fear responding, an inability to extinguish conditioned fear, or both of these factors. An underlying vulnerability factor cannot be considered so, unless it precedes the onset of PTSD (Kremen, Koenen, Afari, & Lyons, 2012). While this presents a particular issue in differentiating consequences from sequelae due to the unpredictable nature of trauma exposure, some studies have examined certain factors in twins discordant for combat-exposure, with some combat-exposed twins being diagnosed with PTSD and others reporting no symptoms. As monozygotic twins share 100% of their genetic makeup, it would be expected that both twins share specific risk factors (both cognitive and biological) with the only differentiating factor being trauma exposure and PTSD diagnosis. Specifically, it would be hypothesized that both twins show similar impairments in fear extinction learning, however trauma exposure mediates the display of PTSD symptoms.

One study has examined fear extinction learning in a monozygotic co-twin design. Milad et al. (2008) measured fear extinction learning and extinction recall in a sample of 14 monozygotic twin pairs discordant for PTSD diagnosis, and combat exposure from the Vietnam War. Seven twin pairs were discordant for PTSD diagnosis and combat exposure, and the remaining seven were only discordant for combat exposure with neither twin diagnosed with PTSD. This study revealed no between-group differences on psychophysiological arousal measures during fear acquisition and extinction recall one day later, compared with their co-twin, or twin pairs discordant for combat exposure. However, the authors revealed that PTSD diagnosis significantly interacts with combat exposure during extinction recall, suggesting that impaired extinction recall is a consequence of

combat exposure, leading to PTSD, rather than a pre-trauma risk factor. As far as we are aware, this idea has not received any attention since.

2.8 Impaired Fear Extinction Learning as a Prospective Risk Factor of PTSD

While trauma-exposure is unpredictable in the general population, researchers have investigated fear extinction learning in people working in high-risk occupations. Despite evidence that impaired fear extinction learning may be a consequence of trauma (Milad et al., 2008), recent evidence has found impaired pre-trauma extinction learning to be a significant predictor of post-traumatic stress symptoms in trainee fire-fighters (Guthrie & Bryant, 2006). Guthrie and Bryant (2006) assessed trainee fire-fighters on PTSD symptoms and diagnosis, and a standardized fear conditioning and extinction task during cadetship (prior to trauma exposure), and reassessed within two years of commencing fire-fighting duties. This study revealed that greater EMG startle responses during extinction (reflecting impaired fear extinction learning) predicted higher PTSD symptoms following trauma exposure (Guthrie & Bryant, 2006). To our knowledge, this is the first study to examine pre-trauma fear extinction as a predictor of PTSD symptoms.

In support of pre-trauma fear extinction impairments predicting greater PTSD (Guthrie & Bryant, 2006), Orr et al. (2012) investigated fear conditioning and extinction in police and fire-fighter recruits. Participants underwent testing during training (prior to trauma exposure), and again several months after job-related trauma exposure. Psychophysiological measures during conditioning and extinction included SCR and electromyogram (EMG) responses to aversive stimuli. Logistic regression showed reduced extinction of forehead EMG responses to be a significant pre-trauma risk factor of greater post-traumatic stress symptoms (Orr et al., 2012). Additionally, at follow-up, psychophysiological assessments were conducted while participants recited the traumatic

experience. Regression identified increased SCR to loud tones as a pre-trauma risk factor for higher physiological reactivity during the recital of the traumatic event (Orr et al., 2012). This second finding indicates that greater psychophysiological reactivity to aversive, or negative stimuli may be a trait-like factor in the development of negative trauma reactions. That is, individuals may have a predisposed tendency to display elevated psychophysiological reactions to aversive or negative stimuli. These findings add further support for the notion that impaired fear extinction learning can predict higher post-traumatic stress symptoms in at-risk populations.

Finally, a recent study extended previous findings to military populations by assessing pre-deployment Dutch military soldiers on a fear conditioning and extinction paradigm (Lommen et al., 2013). Consistent with the aforementioned studies (Guthrie & Bryant, 2006; Orr et al., 2012), Lommen et al. (2013) found impaired fear extinction to be significantly predictive of post-traumatic stress symptoms two months post-deployment to Afghanistan. Extinction performance was assessed by increased US-expectancy ratings to the CS+. A limitation of this study is the absence of a psychophysiological measure of arousal, however US-expectancy ratings are a commonly used measure of associative learning in fear conditioning and extinction paradigms (e.g., Blechert et al., 2007; Norrholm et al., 2011; Sijbrandij et al., 2013), are an externally valid measure of human fear conditioning (Boddez et al., 2013), and do not influence psychophysiological recordings (Blechert, Michael, Williams, Purkis, & Wilhelm, 2008). Therefore, these findings indicate consistent results across several measures of arousal and threat expectancy, with impaired pre-trauma fear extinction as measured using elevated skin conductance response (Orr et al., 2012), elevated EMG corrugator responses (Guthrie & Bryant, 2006), and subjective expectancy ratings (Lommen et al., 2013) found to be predictive of increased PTSD symptoms. While preliminary, these findings provide compelling evidence that an

underlying pre-trauma deficit in extinguishing conditioned fear is an important variable in the etiology of PTSD symptoms.

2.9 Convergent PTSD Risk Factors in Fear Extinction

Here, the present review aims to demonstrate the apparent interactions between PTSD, biomarkers and risk factors, and impaired fear extinction. Previous comprehensive reviews (e.g., Bomyea, Risbrough, & Lang, 2012; Zoladz & Diamond, 2013) have assessed evidence of multiple risk factors of PTSD, concluding there is significant variability due to gender, early trauma history, comorbidity, and dynamic biomarkers. The following sections discuss empirical evidence of the relationship between risk factors (genetics, neuroendocrine system, sex hormones, cognition and neuropsychological factors, and sleep) and PTSD, and their relation to impaired fear extinction learning and memory. It should be noted that due to the breadth of information presented here, specific references to recent reviews are provided where necessary for greater discussions of risk factors that cannot be discussed in the current review.

2.9.1 Genetics.

With advances in understanding DNA methylation and epigenetic influences on gene expression, genomics is claimed to be one of the most promising fields of understanding PTSD susceptibility (Zoladz & Diamond, 2013). While research into genetic risk factors of PTSD is still in its infancy with limited genome-wide association studies, researchers have identified potential candidate genotypes involved in PTSD. Convergent translational findings are emerging from animal, epigenetic, and gene × environment studies in PTSD, focusing specifically on candidate genotypes such as brain-derived neurotrophic factor (BDNF), catechol-O-methyltransferase (COMT), the functional polymorphism of the

serotonin transporter (5-HTTLPR), FK506 binding protein 5 (FKBP5), and pituitary adenylate cyclase-activating polypeptide (PACAP). Furthermore, in many instances, genotype polymorphisms do not solely pose risk for PTSD, but rather interact with environmental factors to increase the likelihood of PTSD development. Bomyea et al. (2012) highlight the importance of studying gene × environment interactions, as stressors during childhood have the potential to reveal biological risk factors, with significant consequences on neural systems.

Research has identified that in some cases, PTSD development can be linked to familial factors (Gilbertson et al., 2006), with approximately one-third of the variability in PTSD symptoms explained by genetic factors (True et al., 1993). Similarly, twin studies have identified that between 35-45% of variability in fear conditioning and extinction can be explained by genetic influences (Hettema, Annas, Neale, Kendler, & Fredrikson, 2003). Research has identified that candidate genotypes play a significant role in the cellular networks of fear memories, and are likely contributors to impaired extinction learning (see Johnson et al., 2012 for a review).

***BDNF*.** BDNF is a member of the neurotrophin family, playing a key role in synaptic plasticity. Specifically, BDNF is concentrated in regions of the central nervous system implicated in PTSD and fear extinction, such as the amygdala, prefrontal cortex, and hippocampus (Rakofsky, Ressler, & Dunlop, 2012). A recent prospective longitudinal study examined serum BDNF levels in motor vehicle accident survivors soon after trauma, and six-months post-trauma (Matsuoka et al., 2013). The results show a positive correlation between serum BDNF levels six-months post-trauma and PTSD severity. Furthermore, the PTSD group displayed significantly higher BDNF levels soon after hospital admission,

indicating that increased BDNF levels soon after trauma could be a biomarker for greater PTSD symptoms.

Matsuoka et al. (2013) identified elevated serum BDNF levels post-trauma as a risk factor for increased PTSD symptoms six-months later. These findings have been supported in cross-sectional research showing higher serum BDNF levels in PTSD relative to controls (Hauck et al., 2009; Hauck et al., 2010). Further, Berger et al. (2010) examined the relationship between PTSD symptoms and serum BDNF over the course of a 12-week treatment program using the SSRI escitalopram. The results of this study show low serum BDNF levels at baseline were associated with greater reduction of PTSD symptoms at the end of treatment. Alternatively, studies have identified lower BDNF levels (serum and plasma) in PTSD compared to trauma-exposed and healthy controls (Angelucci et al., 2014; Dell'Osso et al., 2009). It could be argued, however, that plasma BDNF levels do not accurately reflect cortical levels, as there is evidence that plasma BDNF follows a circadian rhythm, with greater levels early in the day (Begliuomini et al., 2008), and fluctuations with the menstrual cycle in females (Begliuomini et al., 2007).

Translational research shows an important role of BDNF in the fear network. In animal studies, BDNF knock-out mice, characterized by an approximate 50% reduction in BDNF levels, show impaired fear extinction (Psotta, Lessmann, & Endres, 2013), and similarly infusing BDNF into the hippocampus increases neuronal activity in the infralimbic cortex during extinction training, and facilitates extinction learning (Rosas-Vidal, Do-Monte, Sotres-Bayon, & Quirk, 2014). Additionally, Peters, Dieppa-Perea, Melendez, and Quirk (2010) injected BDNF into the infralimbic mPFC of rats, resulting in attenuated fear conditioned responses without undergoing extinction learning.

A single nucleotide polymorphism (SNP) with an amino acid change from a valine to a methionine at position 66 (val66met) in BDNF has recently been implicated in disorders of

fear regulation (Frielingsdorf et al., 2010). Studies have identified carriers of the BDNF val66met met-allele show slow, impaired extinction learning ability (Soliman et al., 2010), and increased vmPFC and amygdala activity during extinction trials (Lonsdorf et al., 2015; Soliman et al., 2010). Further, Felmingham, Dobson-Stone, Schofield, Quirk, and Bryant (2013) identified that PTSD patients with the met66 allele show poorer response to exposure therapy (based on the processes of fear extinction learning) compared to val/val carriers. The role of the val66met SNP in fear extinction has been further reviewed in Notaras, Hill, and van den Buuse (2015).

COMT. COMT is a key enzyme in the prefrontal cortex and hippocampus, working in the degradation of synaptic dopamine levels, as well as epinephrine and norepinephrine (Bomyea et al., 2012). A common SNP coding at position 158 (val158met) is important in the activity of this enzyme. According to Chen et al. (2004), carriers of the val158 allele show increased COMT activity, resulting in greater elimination of dopamine and reduced prefrontal functioning. Alternatively, the met158 allele appears to be associated with reduced COMT activity, and therefore increased synaptic dopamine levels (Tunbridge, Harrison, & Weinberger, 2006). Val/met heterozygotes are believed to demonstrate intermediate levels of COMT activity (Tunbridge et al., 2006), and therefore may show balanced levels of dopamine within the synapse. Therefore, individuals homogenous for either the val or met allele of the COMT gene may be at an increased risk for PTSD, potentially caused by increased or decreased COMT activity in the synapse.

A prospective study by Clark et al. (2013) in members of the United States National Guard deployed to Iraq revealed that homozygous variants of COMT interact with trauma exposure to increase risk for PTSD development. Specifically, val/met allele carriers developed fewer PTSD symptoms under high trauma exposure compared with val/val or

met/met carriers. All participants in this study were white males, potentially limiting generalization to females and other ethnic groups (Clark et al., 2013). Nevertheless, research in Ugandan refugees identified the met/met allele to demonstrate a high risk for PTSD development independent of trauma severity (Kolassa, Kolassa, Ertl, Papassotiropoulos, & De Quervain, 2010). Further, Kolassa et al. (2010) revealed the val/val allele shows a dose-response relationship with trauma exposure to predict PTSD. These findings are supported by research in victims of urban violence, with carriers of the met allele showing increased PTSD symptoms (Boscarino, Hoffman, Rukstalis, & Stewart, 2011; Valente et al., 2011). It is possible that reduced or increased levels of synaptic dopamine degradation in the prefrontal cortex confer risk for PTSD development by inhibiting prefrontal function during trauma exposure.

Risbrough, Ji, Hauger, and Zhou (2014) recently demonstrated that mice with the COMT met/met allele show increased cued fear, and impairments in the recall of extinction memories compared to val-carriers. In humans, met-carriers of the COMT val158met allele have been linked to impaired fear extinction learning. Lonsdorf et al. (2009) found extinction was associated with greater fear-potentiated startle to the CS+ in the met/met genotype, compared to val-carriers. Importantly, the met/met genotype in PTSD has been associated with an impairment to inhibit fear towards the CS-, which represents a safety signal compared to the CS+ (Norrholm et al., 2013; Wendt et al., 2014). Norrholm et al. (2013) demonstrated that performance in fear extinction learning was most impaired in met/met carriers with a current diagnosis of PTSD, compared with trauma-exposed controls. Furthermore, panic disorder patients with the met/met genotype also appear to show little symptom relief from exposure therapy, compared to val-carriers (Lonsdorf et al., 2010), indicating that exposure therapy may not be the most beneficial form of treatment for met-carriers.

Serotonin genes. Selective serotonin reuptake inhibitors (SSRIs) are a commonly used, first line treatment method in PTSD, suggesting altered activity of genes encoding serotonin (5-HT) levels in PTSD (Thakur, Joober, & Brunet, 2009). The functional polymorphism of the serotonin transporter (5-HTTLPR) gene regulates the availability of 5-HT in the synapse, with carriers of the low expression ("short") allele showing reduced 5-HT transcription efficiency compared to the high expression ("long") allele (Lesch et al., 1996). In many instances, specific genotypes may not be directly involved in the etiology of a disorder (medical or psychiatric), but rather interact with environmental influences to increase the likelihood of an individual developing a specific condition. 5-HTTLPR appears to be one such gene, as the short allele of 5-HTTLPR has been shown to interact with trauma exposure and other factors to predict PTSD development.

In the fall of 2004, catastrophic hurricanes on the coast of Florida presented a unique situation for trauma researchers to examine a large population with experience of repeated stressors/traumas. Kilpatrick et al. (2007) interviewed 589 individuals affected by Florida hurricanes regarding hurricane exposure and social support, and collected DNA samples (including the 5-HTTLPR genotype). 5-HTTLPR showed no relationship with PTSD alone, however there was a significant interaction between the short allele, high hurricane exposure and low social support in predicting PTSD diagnosis. This link was not evident in participants with the long allele, low hurricane exposure and high social support. These findings have received further support, indicating that the 5-HTTLPR short allele interacts with environmental stressors to predict PTSD risk, including high crime and unemployment rates (Koenen et al., 2009). In a recent meta-analysis, it was revealed that the 5-HTTLPR short allele had a significant association with PTSD, but only in participants with high trauma-exposure (Gressier et al., 2013). Lee et al. (2005) also found a significantly higher

occurrence of PTSD diagnosis in individuals with two short alleles, compared to carriers of at least one long allele.

A recent study in Israeli Defence Force soldiers involved the collection of prospective data before and during military deployment on the association between attentional threat bias, serotonin genes and PTSD risk (Wald et al., 2013). In opposition to the previous findings, the results show a significant interaction with the combination of high combat exposure, low expression 5-HTTLPR, and attentional threat bias as protective factors for PTSD, rather than risk factors. The relationship between 5-HTTLPR and threat bias may be evident only in military samples, due to attention to threat being considered a normal behavior in infantry soldiers (Wald et al., 2013). Alternatively, Mellman et al. (2009) found no association between 5-HTTLPR and PTSD, however, PTSD was significantly associated with the low expression (G) allele of the 5HT2 receptor antagonist, which is prominent in some pharmacological treatments. This presents an interesting possibility that PTSD risk may be due to the interaction of multiple genes involved in 5-HT activity.

The 5-HTTLPR-s allele has also been associated with a stronger conditioned fear trace. Findings are thus far consistent, with carriers of the 5-HTTLPR-s allele showing significantly stronger startle potentiation during the conditioning phase compared to carriers of the l/l-allele (Hermann et al., 2012; Lonsdorf et al., 2009; although see Lonsdorf et al., 2010). Similarly, studies have replicated these findings in healthy volunteers, but only in carriers of the risk allele (G-allele) for the corticotropin releasing hormone receptor 1 (CRHR1 rs878886; Heitland, Groenink, Bijlsma, Oosting, & Baas, 2013) and the risk allele (T-allele) for the NPS receptor gene (NPSR1 rs324981; Glotzbach-Schoon et al., 2013). These findings indicate that 5-HTTLPR-s interacts with other gene SNPs to promote a biological predisposition for impaired extinction of fear. Wendt et al. (2014) found that carriers of the short and long allele demonstrate comparable fear learning ability, however at

re-conditioning, 5-HTTLPR-s allele carriers showed greater fear potentiated startle levels representing a stronger conditioning trace. These findings posit that carriers of the 5HTTLPR s-allele may develop a conditioning trace that increases in strength, and is resistant to extinction learning.

FKBP5. FKBP5 plays a significant role in the molecular networks of glucocorticoid receptor (GR) sensitivity and translocation, regulating cortisol activity and inhibiting negative feedback activity in the hypothalamic-pituitary-adrenal (HPA) axis (Bomyea et al., 2012). Specific SNPs of FKBP5 have been found to be important in gene × environment interactions, namely: rs9296158, rs3800373, rs1360780, and rs9470080 (Binder et al., 2008; Mehta et al., 2011; Xie et al., 2010). For example, Xie et al. (2010) found that the rs9470080 SNP interacts with early-life trauma to significantly predict PTSD development. Alone, FKBP5 SNPs show no direct effects on PTSD development or symptom severity (Binder et al., 2008; Xie et al., 2010), and appear to only interact with childhood trauma, with no G × E interactions with other forms of trauma (Binder et al., 2008). Research has, however, identified 12-weeks of cognitive behaviour therapy for PTSD is associated with an increase in FKBP5 expression levels (Levy-Gigi, Szabo, Kelemen, & Keri, 2013; Szabo, Kelemen, & Keri, 2014; Yehuda et al., 2013). Wilker et al. (2014) found that carriers of the SNP rs1360780 T allele presented a significant risk of symptom relapse 10-months after exposure-therapy, with non-carriers showing a continuous reduction in symptoms. These findings highlight a significant role for FKBP5 in GR systems following childhood trauma, and in treatment outcome for PTSD.

To our knowledge, only one study has investigated FKBP5 in fear extinction and the retention of fear. Sawamura et al. (2016) recently used an animal model of PTSD to examine dexamethasone in extinction learning, and retention of extinction one day later. The authors

found dexamethasone to be associated with a dose-dependent enhancement of extinction learning and retention. Furthermore, dexamethasone treatment was associated with reduced FKBP5 mRNA expression in the amygdala after extinction learning and retention. It was suggested that dexamethasone worked to enhance extinction learning and retention via dynamic FKBP5 regulation in the HPA axis. In support, the glucocorticoid receptor is critical in managing the stress response, and it is believed that the glucocorticoid receptor is regulated via the FKBP5 gene (Zannas & Binder, 2014).

PACAP-PAC$_1$. PACAP is a protein encoded by the ADCYAP1 gene, and important in the activation of stress circuitry within the central and peripheral nervous systems (Uddin et al., 2013). The ADCYAP1 and ADCYAP1R1 (responsible for encoding the PAC_1 receptor) genes have recently been associated with sex-specific PTSD risk in women, but not men. Ressler et al. (2011) found that a single SNP (rs2267735) within the ADCYAP1R1 gene was a significant predictor of PTSD diagnosis and posttraumatic stress symptoms in highly traumatized African American women. A follow-up study by Almli et al. (2013) supported these findings with a significant genotype × trauma interaction, showing that the combination of ADCYAP1R1 gene and high trauma exposure predicted PTSD. Further, while Uddin et al. (2013) found no evidence of a direct effect of ADCYAP1R1 on PTSD development, they did find that ADCYAP1R1 interacts with high levels of childhood maltreatment to promote PTSD risk. Samples with relatively lower trauma-exposure have failed to find an association between the gene and PTSD (Chang et al., 2012), suggesting an underlying risk factor in the presence of high trauma exposure. On the basis of these preliminary findings, it is possible that the PACAP-PAC_1 pathway (and associated genes) may be used to confer risk for increased PTSD symptoms in women with increased trauma-

exposure, however prospective longitudinal studies are required to test this hypothesis (Dias & Ressler, 2013).

Ressler et al. (2011) investigated mRNA changes in the ADCYAP1R1 allele of PACAP in the mouse brain during fear conditioning. The results show a significant increase in the amygdala ADCYAP1R1 mRNA during conditioning, with a similar relationship in the mPFC. Recently, Schmidt et al. (2015) infused PACAP-38 into the CA1 region of the hippocampus or the BLA of rats prior to a contextual fear extinction task. The results indicated that PACAP-38 infused in the amygdala resulted in a significantly greater percentage of freezing during a recall test (24 hours after extinction learning). These results indicate that fear acquisition processes in the amygdala and consolidation/retrieval processes in the mPFC (Myers & Davis, 2007) and contextual extinction recall in the hippocampus (Schmidt et al., 2015) may be moderated by PACAP expression in these structures. Translating these findings to humans would further clarify the role of PACAP in fear memory.

Summary. Research in biomarkers of PTSD, and particularly genetic biomarkers, has been claimed to be the most promising field of advancing our understanding of PTSD risk (Zoladz & Diamond, 2013). Prospective and cross-sectional research has consistently linked increased serum BDNF to greater PTSD symptoms, and the BDNF val66met met-allele is associated with poorer therapeutic and experimental extinction learning. Homozygous variants of the COMT gene (i.e., met/met and val/val carriers) are associated with greater PTSD risk and significantly poorer fear extinction learning. There is consistent evidence that the 5-HTTLPR-s allele interacts with environmental stressors and high trauma-loads to increase risk for PTSD, and the s-allele is associated with stronger fear acquisition, however research in fear extinction is currently limited. FKBP5 SNPs appear to

interact specifically with childhood trauma to promote PTSD risk, and dexamethasone administration enhances extinction learning and retention via FKBP5 mRNA regulation in the HPA axis. Research is currently consistent that PACAP-encoding genes interact with high-stress environments to promote PTSD risk, and animal research shows PACAP administration to the rat amygdala leads to a reduction in fear extinction recall.

2.9.2 Neuroendocrine system.

As PTSD is a disorder primarily characterized by increased anxiety, stress and arousal, a logical assumption is that this disorder may involve irregularities in cycling hormones associated with the stress response. Specifically, stress hormones (i.e., cortisol and noradrenaline) are critical in the fear network and regulating fear responding. This assumption has led researchers to investigate neuroendocrine functioning in PTSD. Understanding neuroendocrine abnormalities in PTSD carries important implications for pharmacological treatments (for a comprehensive review on the pharmacological enhancement of exposure therapy, see Singewald, Schmuckermair, Whittle, Holmes, & Ressler, 2015).

Hypothalamic-pituitary-adrenal (HPA) axis and cortisol. The HPA axis is the mammalian brain's premier neuroendocrine centre, which acts to regulate the release of hormones in different situations, including stress. Following stress exposure, the hypothalamus secretes a number of hormones and neuropeptides that stimulate the release of adrenocorticotropic hormone (ACTH) from the pituitary. ACTH facilitates cortisol release from the adrenal glands to promote a physiological environment for accommodating a stressful event. Cortisol release acts in a negative feedback loop to return the HPA axis to homeostasis in preparation for any subsequent activation (Munck & Guyre, 1986).

The assumption that the HPA axis is particularly sensitive to stressful responding in some situations has led to extensive research on the relationship between cortisol and PTSD diagnosis (Bomyea et al., 2012). With cortisol levels increasing synonymously with stress, investigators believed that PTSD might be associated with hyperactivity of naturally cycling cortisol levels (Zoladz & Diamond, 2013). Rather, PTSD has been associated with lower basal cortisol levels. Yehuda et al. (2009) found that after roughly 14 sessions of psychotherapy, cortisol levels differentiated non-responders to treatment from responders in survivors of the September 11 terrorist attack, with non-responders displaying reduced urinary cortisol levels. These findings indicate that lower cortisol levels may be used as a marker of treatment-resistant PTSD. Some studies have found results to support the notion that PTSD is associated with lower levels of naturally cycling cortisol levels compared to trauma-exposed and healthy controls (Bicanic et al., 2013; Wahbeh & Oken, 2013; Yehuda et al., 2007; although see Groer, Kane, Williams, & Duffy, 2014; Shalev et al., 2008).

It is important to understand whether cortisol levels (a reflection of HPA axis activity) are an underlying biological risk factor for PTSD, or a product of trauma exposure (McFarlane, Atchison, & Yehuda, 1997), and hence require prospective investigation. A recent longitudinal study examined salivary cortisol levels in the morning and afternoon following hospital admission in 48 traumatic accident survivors (McFarlane, Barton, Yehuda, & Wittert, 2011). PTSD symptomatology was assessed six months post-trauma, revealing a significant, negative correlation between morning cortisol levels and PTSD symptoms six months later. Interestingly, afternoon cortisol levels were positively correlated with PTSD symptoms. These findings are in partial support of previous longitudinal studies in adults showing low peri-trauma cortisol levels to predict PTSD symptomatology at follow-ups (Delahanty, Raimonde, & Spoonster, 2000; McFarlane et al., 1997), and that cortisol increases in the remission of PTSD symptoms in Holocaust survivors (Yehuda,

Morris, et al., 2007). Interestingly, a prospective study in children found a sex-specific relationship between increased peri-trauma cortisol levels and increased PTSD symptoms six weeks later in boys, but not girls (Delahanty, Nugent, Christopher, & Walsh, 2005), thus presenting an interesting question regarding the differential effects of trauma exposure on the maturing sympathetic nervous system and HPA axis.

The above findings note some important implications for future research, notably regarding the inconsistency surrounding lower versus higher cortisol levels in PTSD samples, and in prospective prediction of PTSD. Indeed, some of these inconsistencies may be grounded in wide methodological variations in the collection and subsequent analysis of hormone samples (i.e., differences between blood, urine, and salivary collection methods). In addition, time-of-day is an important factor to take into account when measuring cortisol, which may explain differing morning versus afternoon levels in the recent prospective study by McFarlane et al. (2011). That is, cortisol experiences a surge shortly after waking, known as the cortisol awakening response (van Zuiden et al., 2011), which may bias findings.

Research suggests that increased cortisol enhances the formation of extinction memories (Bentz et al., 2013; Merz, Hamacher-Dang, & Wolf, 2014; Pace-Schott et al., 2013), although these findings are somewhat mixed (see Raio, Brignoni-Perez, Goldman, & Phelps, 2014; Tabbert et al., 2010). Using a contextual fear conditioning task, Merz et al. (2014) found that following stress-induction (promoting an increase in cortisol levels), participants displayed reduced fear responses in the extinction context and attenuated responses in the acquisition context. Alternatively, Raio et al. (2014) found that following an acute stress task, participants displayed significantly worse recall of extinction memory compared to those who did not undergo the stress task. Evidence for the role of cortisol in the consolidation of extinction memories is somewhat mixed.

Studies have also examined the effect of acute stress tasks and cortisol administration on the efficacy of exposure-based treatments for specific phobias. Soravia et al. (2006) investigated oral cortisol consumption and response to exposure therapy in a sample of participants with a spider phobia. The results indicate that cortisol led to a reduction in spider fear that was maintained at retest two days later, compared to placebo treatment (Soravia et al., 2006). These findings suggest that cortisol administration facilitated and enhanced fear extinction learning, which has received further support in phobias of heights (de Quervain et al., 2011) and spiders (Soravia et al., 2014). To our knowledge, research has not yet examined these effects in a clinical PTSD sample.

Noradrenaline. Evidence suggests that noradrenaline (NA) interacts with cortisol during the presentation of emotional stimuli to enhance the consolidation of emotional memories (for a review, see McGaugh, 2004). Noradrenaline is a catecholamine playing important hormonal and neurotransmitter functions during the "fight or flight" stress response. Rat models of posttraumatic stress have revealed exaggerated NA utilisation levels in the locus coeruleus following stress (George et al., 2013), and increased NA levels in the rostral pons were related to the presence of trauma reminders (Terzioglu et al., 2013). In clinical studies in humans, PTSD has been linked to increased salivary, urinary, blood, and cerebrospinal fluid NA levels compared to trauma-exposed and healthy controls (Geracioti et al., 2001; Kosten, Mason, Giller, Ostroff, & Harkness, 1987; Nicholson, Bryant, & Felmingham, 2014; Pietrzak et al., 2013; Yehuda et al., 1998; Yehuda, Southwick, Giller, Ma, & Mason, 1992). A longitudinal study by Videlock et al. (2008) measured NA levels in emergency room admissions and again at 10 days, 1-month, and 5-months post-admission. Participants who developed PTSD at 5-months had significantly lower plasma NA levels at

10 days, 1-month, and 5-months post-trauma, contradicting findings of increased NA levels in cross-sectional research.

An additional method of measuring the impact of NA on PTSD symptoms is via pharmacological challenge studies with agents known to impact NA levels. Yohimbine and prazosin are two such drugs that have been assessed in PTSD. Yohimbine is an α-$_2$ adrenergic receptor antagonist that increases NA levels (Southwick, Morgan Iii, Charney, & High, 1999), while prazosin is an α-$_1$ adrenergic receptor antagonist that reduces NA levels (Fuller, Snoddy, & Perry, 1978). Studies of yohimbine administration have shown an increase in PTSD re-experiencing symptoms (Southwick et al., 1999). Prazosin administration, however, has been associated with a significant decrease in PTSD-related sleep disturbances and nightmares (Raskind et al., 2007; Raskind et al., 2003), a significant increase in mean REM duration (Taylor et al., 2008), and a significant reduction of PTSD symptom severity and psychological distress (Raskind et al., 2003; Taylor et al., 2006; Taylor et al., 2008). Further, propranolol administration (a β-adrenergic receptor antagonist) appears to block the effects of stress (Zoladz & Diamond, 2013), and shows promise in the treatment of PTSD (Brunet et al., 2011; Pitman, 2011; Pitman et al., 2002; Vaiva et al., 2003). These findings indicate that hyper-activity of the noradrenergic system, particularly NA release, is associated with greater severity of PTSD symptoms, and the blockade of NA release may be an effective strategy in PTSD treatment. To our knowledge, research has not yet investigated pre-trauma NA levels, and how these may interact with trauma and epigenetic processes to increase or reduce risk for PTSD.

In regards to fear extinction, mice that receive a post-extinction injection of epinephrine show an impaired ability to recall extinction learning, unless another injection is received immediately prior to extinction recall (Rosa, Myskiw, Furini, Sapiras, & Izquierdo, 2014). Additionally, increased NA into the amygdala of rats enhanced extinction learning

(Berlau & McGaugh, 2006). Thus far, findings appear consistent, with greater NA levels influencing greater beta-receptor signaling which, in turn, strengthens memory formation (Mueller & Cahill, 2010). Greater activation of noradrenergic receptors during fear extinction is associated with better retrieval of extinction (Mueller, Porter, & Quirk, 2008). In further support, stimulation of noradrenergic beta-receptors in the amygdala following the retrieval of conditioned responses results in a stronger memory trace that is resistant to extinction (Debiec, Bush, & LeDoux, 2011). These results posit that whether conditioning or extinction is being learned, enhanced noradrenergic receptor signaling results in a stronger memory trace.

A recently expanding body of literature is administering an α_2-adrenergic receptor antagonist (i.e., yohimbine) and a β-adrenergic receptor antagonist (i.e., propranolol) at select intervals during a fear conditioning paradigm to investigate their effects on reconsolidation and extinction learning. Thus far, studies are somewhat mixed with some studies showing that after fear acquisition, the use of propranolol attenuates fear responses when administered in close proximal timing with the conditioned stimuli (Kindt, Soeter, & Sevenster, 2014; Soeter & Kindt, 2012) while others note impairments in extinction learning (Bos, Beckers, & Kindt, 2012, 2014; Soeter & Kindt, 2011). Further, Soeter and Kindt (2012) administered yohimbine immediately prior to fear acquisition, leading to an increase in NA release, and a fear conditioning trace that was broadly generalized and resistant to extinction. Importantly, the aim of these studies was to target the reconsolidation window for the original memory trace to prevent the return of fear (Kindt et al., 2014) rather than the formation of a new memory during extinction that is likely in constant competition with the original fear memory.

Summary. While the evidence suggests that PTSD is associated with hyperactive HPA axis activity (via reduced cortisol release, and therefore lower negative feedback of the HPA axis stress response), studies are inconsistent regarding the influence of cortisol levels (lower versus higher) on fear extinction learning and memory. Cross-sectional research suggests that PTSD is linked to increased saliva, urinary, blood, and cerebrospinal fluid levels of NA, however a longitudinal study found lower peri-trauma plasma NA levels predicted PTSD symptoms 5-months post-trauma. Further, the pharmacological enhancement of noradrenergic signaling during extinction learning appears to strengthen extinction. While evidence indicates that cortisol and noradrenaline are involved in PTSD symptoms, and the regulation of fear responding, this research field appears to be marred by certain inconsistencies that require clarity.

2.9.3 Sex hormones: Estrogen and progesterone

Women are twice as likely to develop PTSD compared to men (Glover, Jovanovic, & Norrholm, 2015; Kessler et al., 1995; Shansky, 2015) despite higher rates of trauma exposure in men. McLean, Asnaani, Litz, and Hofmann (2011) showed greater lifetime PTSD rates in women compared to men in the United States (8.5% vs. 3.4% respectively). Due to this increased prevalence in women compared to men, there has been a recent growth in research investigating sex differences in PTSD. In their review, Zoladz and Diamond (2013) highlight that greater PTSD risk in women is not considered to be due to factors such as trauma type, but may be due to biological differences, an idea that has been previously discussed by Cahill (2006). A recent prospective study found that women in the luteal phase of their menstrual cycle at the time of trauma were significantly more likely to experience flashbacks of the trauma compared to other women (Bryant et al., 2011). The luteal phase of the menstrual cycle is associated with an increase of estrogen and progesterone levels. In

tandem with increasing sex hormones, the luteal phase involves an increase in glucocorticoid release (Handa, Burgess, Kerr, & O'Keefe, 1994), which is associated with greater emotional memory consolidation (Abercrombie, Kalin, Thurow, Rosenkranz, & Davidson, 2003). Bryant et al. (2011) hypothesize that greater consolidation of trauma memories may lead to an increase in flashback memories. Indeed, additional research has linked increased estrogen and progesterone levels, and the luteal phase with greater intrusive memories (Cheung, Chervonsky, Felmingham, & Bryant, 2013; Ferree, Kamat, & Cahill, 2011; Soni, Curran, & Kamboj, 2013) and greater recall of threatening images (Felmingham, Fong, & Bryant, 2012). This evidence has implications for the menstrual cycle and associated hormone fluctuations as a key mechanism in the development of PTSD symptoms in women, but not men.

Considerable research examining sex differences in PTSD and disorders characterized by excessive fear has stimulated research examining sex differences in fear extinction (for a comprehensive review, see Lebron-Milad & Milad, 2012). Milad et al. (2010) investigated the effects of estradiol in a two-day fear extinction learning and recall paradigm. Healthy men and women underwent fear acquisition and extinction on day one and extinction recall on day two. Women with low levels of estradiol had significantly poorer extinction recall on day two, compared to women with higher levels of estradiol. Men had comparable extinction recall to high-estradiol women, which may speculatively be attributed to increased testosterone levels in men (Milad et al., 2010), although more research is needed on the relationship between testosterone and fear extinction. Further, Wegerer, Kerschbaum, Blechert, and Wilhelm (2014) recently found that healthy women with low estradiol demonstrate poorer fear extinction and more intrusive memories than women with higher estradiol. These findings support previous research indicating that higher estrogen levels result in stronger consolidation of fear extinction learning in rodents

(Milad, Igoe, Lebron-Milad, & Novales, 2009) and humans (Glover et al., 2013; Milad et al., 2010; Zeidan et al., 2011). Further, Graham and Milad (2013) investigated the effects of hormone contraceptives on fear extinction learning in female rats and healthy women. The use of contraceptives results in a reduction of estrogen levels and, consistent with previous research, was associated with significantly impaired fear extinction recall. Importantly, women with PTSD and low estrogen levels show impaired fear extinction learning, compared with trauma-exposed controls with low estrogen levels (Glover et al., 2012), suggesting that other factors are involved.

With estrogen levels naturally fluctuating in women, researchers have identified that specific phases of the menstrual cycle may confer greater risk for poor consolidation of extinction memories. Recently, Glover et al. (2013) measured extinction recall in a sample of women currently in the follicular phase (lower estrogen) versus the luteal phase (high estrogen). The results indicated that the follicular phase was associated with poorer recall of extinction memories. Furthermore, lower estradiol levels in naturally cycling premenopausal women have been associated with lower extinction recall (Zeidan et al., 2011). Studies in rodents similarly found the proestrus menstrual phase (characterized by high estrogen/progesterone) to be associated with greater consolidation of extinction learning (Milad, Igoe, et al., 2009). Interestingly, Milad, Goldstein, et al. (2006) found that women in the late follicular phase (high estrogen) to show poorer extinction recall than early follicular phase women and men. While these findings go against the results of previous studies, the authors highlight that this menstrual phase is associated with lower activation of the vmPFC, which may account for poorer extinction recall due to the role that this structure plays in the consolidation and retrieval of extinction memories (Mueller & Cahill, 2010).

2.9.4 Cognitive factors.

PTSD is reliably associated with deficits in attention and memory function, with impairments on these faculties included as diagnostic criteria of the DSM-IV (American Psychiatric Association, 2000; Vasterling & Brailey, 2005). Furthermore, PTSD has previously been characterized as a disorder of memory (McNally, 2006) due to hallmark re-experiencing symptoms such as distressing intrusive memories, and deficits in episodic and autobiographical memory. Longitudinal studies in combat veterans have identified that PTSD diagnosis at post-deployment has been associated with significant pre-deployment cognitive deficits in intellectual functioning, attention, learning, and memory (Gale et al., 2008; Kremen et al., 2007; Marx, Brailey, et al., 2009; Marx, Doron-Lamarca, Proctor, & Vasterling, 2009; Thompson & Gottesman, 2008; Vasterling et al., 2012; Vasterling et al., 2006). Despite overwhelming and consistent evidence of cognitive deficits in PTSD, little research has investigated the relationship between cognitive factors and fear extinction. Due to this, here we review evidence of specific PTSD-related cognitive functioning in the areas of intellectual functioning, attention, and memory, followed by a section discussing the currently limited research in fear extinction and cognitive factors.

Intellectual functioning. While the measurement of "intelligence" in PTSD has been met with skepticism (Vasterling & Brailey, 2005), a number of studies have implicated a role for lower intellectual functioning in the development of PTSD (Breslau, Chen, & Luo, 2013; Gilbertson, Gurvits, Lasko, Orr, & Pitman, 2001; Macklin et al., 1998; Vasterling, Brailey, Constans, Borges, & Sutker, 1997; Vasterling et al., 2002). A study in monozygotic twin pairs discordant for combat exposure and PTSD diagnosis indicated that lower intelligence was a familial risk factor for greater PTSD symptom severity (Gilbertson et al., 2006). Specific subscales of intelligence tests have identified verbal intellectual performance

to be specifically impaired in PTSD (Vasterling & Brailey, 2005). Studies in children have revealed that those with PTSD show significantly lower verbal IQ on the WISC-III (Saigh, Yasik, Oberfield, Halamandaris, & Bremner, 2006; Samuelson, Krueger, Burnett, & Wilson, 2010). Further, Saigh et al. (2006) found no significant differences on performance IQ, with effects limited to verbal subtests (i.e., vocabulary and comprehension). These results have been supported in a study of combat veterans of the Gulf War, with poor performance on the WAIS-R being limited to verbal IQ in veterans with PTSD compared to veterans without PTSD (Vasterling et al., 1997). Additional research has identified verbal intellectual function (including verbal learning and memory) to be a primary cognitive complaint in PTSD (Barrett, Green, Morris, Giles, & Croft, 1996; Bremner, Vermetten, Afzal, & Vythilingam, 2004; Gilbertson et al., 2006; Grigorovich, Gomez, Leach, & Fish, 2013; Jelinek et al., 2006; Marx, Doron-Lamarca, et al., 2009; Parslow & Jorm, 2007; Samuelson et al., 2010; Vasterling et al., 2002).

Attention. Studies have identified that PTSD is associated with deficits in attentional resources. Recently, Flaks et al. (2014) found differences in processes of selective attention and short-term working memory capacity in a sample of urban violence victims with PTSD compared to trauma-exposed and non-exposed comparison groups. Patients with PTSD show significantly poorer performance than healthy controls on the digit span test, which is a measure of sustained attention and short-term memory (Horner, Mintzer, Turner, Edmiston, & Brawman-Mintzer, 2013). The findings of these studies are supported by numerous studies revealing PTSD-related attentional deficits in children (Beers & De Bellis, 2002; Samuelson et al., 2010) and veterans (Gilbertson et al., 2006; Marx, Brailey, et al., 2009; Toomey et al., 2009; Vasterling et al., 2002; Vasterling et al., 2006). However, null findings of attentional deficits have been revealed in a community sample (Crowell, Kieffer,

Siders, & Vanderploeg, 2002) and well-educated combat veterans with PTSD (Neylan et al., 2004).

With hyperarousal to threat being a key diagnostic symptom of PTSD (American Psychiatric Association, 2013), studies have investigated attention biases to threatening stimuli in PTSD. Prospective studies have identified attentional biases to threat are significant predictors of PTSD development in combat veterans (Wald et al., 2013) and motor vehicle accident survivors (Naim et al., 2013). Specifically, Wald et al. (2013) identified attentional threat biases at military recruitment (pre-trauma) to be a significant predictor of post-combat PTSD symptoms. These findings have been supported in cross-sectional studies indicating that veterans with PTSD show sustained attention to threatening stimuli, compared to comparison control groups (Armstrong, Bilsky, Zhao, & Olatunji, 2013; Olatunji, Armstrong, McHugo, & Zald, 2013).

Memory. Impaired attention in PTSD carries important implications for memory processes. As with verbal intelligence being the most consistent deficit in studies of PTSD-related intellectual functioning, a meta-analysis revealed verbal memory processes to be more consistently impaired in PTSD than nonverbal processes (Brewin, Kleiner, Vasterling, & Field, 2007). Improvements in verbal learning and memory have been associated with a reduction in PTSD symptom severity (Yehuda et al., 2006). Alternatively, a study by Wild and Gur (2008) found that poor response to eight sessions of cognitive behavioral therapy in PTSD was significantly predicted by poor verbal memory. These results are supported by growing research showing verbal learning and memory to be significantly impaired in PTSD (Barrett et al., 1996; Bremner et al., 2004; Gilbertson et al., 2006; Grigorovich et al., 2013; Jelinek et al., 2006; Marx, Doron-Lamarca, et al., 2009; Parslow & Jorm, 2007; Samuelson et al., 2010; Vasterling et al., 2002).

In a recent prospective study, Marx, Doron-Lamarca, et al. (2009) assessed neurocognitive functioning in a sample of pre-deployed U.S. Army soldiers, and assessed PTSD symptom severity at post-deployment. The results showed pre-deployment immediate visual recall was negatively correlated with PTSD symptoms at post-deployment (Marx, Doron-Lamarca, et al., 2009). Patients with PTSD have also shown poorer performance on working memory (Toomey et al., 2009; Vasterling et al., 2002), and visual recall memory (Samuelson et al., 2009). Further, veterans with chronic PTSD demonstrate poorer memory performance than veterans without PTSD (Yehuda, Golier, et al., 2007), which may be a consequence of prolonged secretion of stress hormones (Bomyea et al., 2012).

Fear extinction and cognitive factors. Despite consistent, and accumulating evidence of cognitive deficits in PTSD, the relationship between cognitive factors and fear extinction is a relatively new field. A recent study found that greater cognitive load during fear extinction resulted in impaired fear extinction learning (Raes et al., 2009). That is, preliminary evidence indicates that fear extinction learning relies on sufficient cognitive resources for efficient consolidation of extinction memories (akin to a dual-processing approach). The aforementioned cognitive processes of intelligence, attention, memory, and verbal functioning are impaired in PTSD, suggesting that these cognitive resources may be involved in fear extinction.

This notion—that fear extinction learning is a cognitive process relying on the use of multiple cognitive faculties—has received little attention, yet some relationships have been identified. Fear acquisition shares similarities to an attentional threat bias (Fani et al., 2012; Van Damme, Crombez, Hermans, Koster, & Eccleston, 2006), with attentional threat biases increasing, decreasing, and returning in parallel with acquisition, extinction, and reinstatement phases of a differential fear conditioning paradigm (Van Damme et al., 2006).

Additionally, Gazendam and Kindt (2012) found that engaging with a verbal task led to impaired fear extinction learning. Together, these early findings provide preliminary support for the idea that fear extinction is not an automatic process, but relies on cognitive resources for accurate consolidation (Raes et al., 2009). Fear extinction and generalization are inherently learning and memory processes, requiring the accurate consolidation and recall availability to reduce the likelihood of reinstatement or spontaneous recovery of conditioned fear responses (e.g., Milad & Quirk, 2012). Based on the research so far discussed, multiple factors affect memory processes required for the storage or generalization of extinction memories, however to our knowledge, a link between memory capacity and fear extinction learning ability has not yet been examined.

Summary. Studies in PTSD populations have consistently revealed lower levels of intelligence, attention, and memory to be associated with the disorder. Specifically, lower verbal intelligence, as well as verbal learning and memory appear to be primary complaints of the disorder. While still in its infancy, investigations of fear extinction and cognitive factors indicate that fear extinction ability is significantly hindered by increasing cognitive load, or by engaging in a verbal cognitive task. Therefore it can be assumed that fear extinction learning relies on sufficient cognitive resources for accurate consolidation, and the processing of an additional task reduces cognitive resources available for the consolidation of extinction.

2.9.5 Sleep disturbances.

Sleep disturbances are widely considered to be a hallmark symptom of PTSD (Germain, 2013; Ross, Ball, Sullivan, & Caroff, 1989), and meet specific DSM-5 criteria relating to alterations in arousal and intrusive memories (American Psychiatric Association, 2013). Sleep is argued to be a central stage involved in emotional memory consolidation

(reviewed in Pace-Schott et al., 2015a), and rapid eye movement (REM) sleep has been implicated in the consolidation of extinction memories (Spoormaker et al., 2010). Indeed, some studies have identified significant differences in REM sleep between clinical PTSD samples and comparison groups (Engdahl, Eberly, Hurwitz, Mahowald, & Blake, 2000; Mellman, Kumar, Kulickbell, Kumar, & Nolan, 1995; Mellman, Nolan, Hebding, KulickBell, & Dominguez, 1997; Ross et al., 1994a, 1994b), while other studies have revealed small to no differences (Breslau et al., 2004; Hurwitz, Mahowald, Kuskowski, & Engdahl, 1998). Importantly, studies examining specific sleep architecture disturbances in PTSD have revealed variable findings in relation to the specific disturbances in sleep stages (i.e., differences in REM density, duration and frequency; for a comprehensive review, see Pace-Schott, Germain, & Milad, 2015b).

Prospective studies have found that objective and subjective sleep disturbances predict the development of PTSD. An early study tracked PTSD development and sleep difficulties of motor vehicle accident survivors over the course of one year (Koren, Arnon, Lavie, & Klein, 2002), finding increased excessive daytime sleepiness and insomnia symptoms at one month post-trauma significantly predicted PTSD development 11-months later. Furthermore, this difference became larger over the following 11-months, with the PTSD group reporting significantly poorer sleep quality. Additional studies have also found sleep disturbances at the time of the trauma to be a significant predictor of future PTSD diagnosis (Bryant et al., 2010; Mellman, Bustamante, Fins, Pigeon, & Nolan, 2002; Mellman & Hipolito, 2006; Mellman, Knorr, Pigeon, Leiter, & Akay, 2004; Mellman, Pigeon, Nowell, & Nolan, 2007).

Nightmares are one of the most common symptoms reported in PTSD as a form of intrusive memory (Germain, Buysse, & Nofzinger, 2008; Kobayashi, Boarts, & Delahanty, 2007; Levin & Nielsen, 2007). Pre-deployment nightmares have been found to predict

PTSD development in soldiers (van Liempt, van Zuiden, Westenberg, Super, & Vermetten, 2013), and a therapeutic reduction in nightmares using imagery rehearsal therapy led to an improvement in PTSD symptoms (Krakow et al., 2001). Together, these findings indicate that sleep disturbances may be an important variable in the maintenance of PTSD symptoms, and are an important factor to be considered during treatment.

There is considerable evidence that sleep quality is important for memory consolidation (Stickgold, 2005). The consolidation of fear extinction learning also requires sufficient sleep quality, with an emphasis on REM sleep (for a review, see Pace-Schott et al., 2015a). While only one study has identified impaired extinction learning in REM-deprived rats versus controls (Silvestri & Root, 2008), REM deprivation appears to consistently limit the consolidation of extinction learning, as evidenced by impaired extinction recall and/or generalization (Fu et al., 2007; Pace-Schott, Verga, Bennett, & Spencer, 2012; Spoormaker et al., 2012). Participants deprived of sleep show greater SCR to the extinguished stimulus (Spoormaker et al., 2010), and poor generalization from an extinguished CS+, to an unextinguished CS+ (Pace-Schott et al., 2009). Recently, Spoormaker, Gvozdanovic, Samann, and Czisch (2014) found greater left vmPFC activity during fear conditioning to be associated with lower physiological expressions of fear during extinction learning the following day. Furthermore, greater left vmPFC activity was positively associated with REM sleep amount, which was also negatively associated with fear expression during extinction. The authors concluded that left vmPFC activity during fear conditioning is associated with greater extinction learning, and that this relationship is mediated by REM sleep quality (Spoormaker et al., 2014). This idea shows parallels with the finding that regions of the prefrontal cortex play specific roles in the consolidation of acquisition, extinction learning, and recall of extinguished fear memories (Milad, Wright, et al., 2007).

The aforementioned findings have important implications for the simultaneous treatment of sleep disturbances and avoidance symptoms in anxiety disorders. That is, there is some interplay between extinction learning and sleep quality, with each factor having enhancing effects on the other. So, while sleep appears to promote better consolidation of extinction memories (Pace-Schott et al., 2009), extinction training also appears to improve sleep quality (Sturm, Czisch, & Spoormaker, 2013). These findings are supported by research in clinical populations showing that sleep following exposure therapy results in significant symptom reductions in spider phobia (Kleim et al., 2014). Furthermore, sleep disturbances have been improved with exposure therapy in clinical PTSD samples (Gutner, Casement, Stavitsky Gilbert, & Resick, 2013; Long et al., 2011) and cognitive therapy for PTSD (Lommen et al., 2016). Despite these findings, however, Lommen et al. (2016) found that sleep quality and duration did not predict long-term treatment outcome in PTSD patients, and Datta and O'Malley (2013) found that greater sleep quantity is insufficient for extinction consolidation. Specifically, extinction learning was only recalled if post-extinction training REM sleep contained pontine-wave activity in the brainstem. These results show important implications for the quality of REM sleep in the consolidation of extinction memory, rather than the quantity of REM sleep. Furthermore, these findings suggest an important role of the brainstem in extinction memory consolidation (Datta & O'Malley, 2013). In summary, the role of sleep plays an integral role in the persistence of conditioned fear and consolidation of extinction memory, and should be paid close attention in the etiology and treatment of anxiety disorders.

Summary. Prospective studies are consistent that pre- and peri-trauma sleep disturbances predict future PTSD symptoms. Evidence is also consistent that sleep deprivation leads to poor extinction learning and generalization, with mixed findings for the

role of physiological sleep stages, such as REM sleep. Further, clinical studies have shown that sleep disturbances and PTSD symptoms are reduced in tandem by exposure therapy and CBT for PTSD.

2.10 Summary and Conclusions

The research presented in this review suggests that risk factors of PTSD may also share links with fear extinction. Although we acknowledge some inconsistences in the literature, the balance of evidence supports the notion that impaired fear extinction is a fundamental factor in PTSD etiology that is involved in, or influenced by, these additional risk factors. Substantial evidence shows impaired extinction learning, recall, and the generalization of extinction in PTSD populations compared to trauma-exposed and non-exposed groups, in addition to serving as a pre-trauma risk factor. The over-expression of conditioned fear during the early stages of extinction learning (termed fear load) has been significantly associated with the presentation of intrusive thoughts of trauma-related fear memories, and is argued to present a quantifiable intermediate phenotype underlying the etiology of fear-related psychopathologies (Norrholm et al., 2015). The extinction of a learned fear association is also the key concept driving exposure therapy, the first line treatment approach for PTSD and specific phobia. On this basis, the identification of fear extinction as a key variable of PTSD development provides specific clinical relevance, as well as theoretical relevance. Further enhancing our understanding of the processes of fear extinction can greatly improve our understanding of PTSD risk, and whether extinction learning potential can be improved for the purpose of preventing exaggerated fear responses that lead to significant health consequences.

Across a number of research fields, including genetics, hormonal (both stress and sex hormones), cognitive, and sleep disturbances, research is revealing important links between

PTSD risk factors and fear extinction (see Table 1 for a concise summary of the relationship between risk factors and PTSD, and between risk factors and impaired fear extinction/response to exposure-based therapies). For example, women with low estrogen levels show significantly poorer fear extinction recall compared to women with high estrogen levels, and men (Milad et al., 2010), and that menstrual phase at the time of trauma is a significant risk factor for PTSD (Bryant et al., 2011). Similarly, verbal intelligence and memory are the most consistently impaired cognitive functions in PTSD (Brewin et al., 2007), and evidence indicates that extinction learning is impaired when individuals are required to perform tasks utilizing verbal functioning (Gazendam & Kindt, 2012). Disturbed sleep is considered a hallmark symptom of PTSD (Germain, 2013), and growing evidence is implicating greater sleep quality to be a key mechanism in healthy extinction learning, recall, and generalization (Pace-Schott et al., 2015a). Indeed, recent reviews have acknowledged the important interaction between disturbed sleep and impaired fear extinction learning in the etiology of anxiety disorder symptoms (Pace-Schott et al., 2015a, 2015b). The aforementioned factors are just a select few presented in this review to support the contribution that impaired fear extinction processes play in PTSD etiology, with significant theoretical and clinical implications.

Table 1

Risk Factors of PTSD, and their Relationship with Impaired Fear Extinction/Exposure Therapy.

Risk factor	Relation to PTSD	Relation to impaired fear extinction/exposure therapy
Genotypes		
- BDNF	Increased BDNF levels soon after trauma predict greater PTSD	Val66met associated with slow (impaired) extinction learning.

	symptoms.	Val66met associated with reduced response to exposure therapy.
- COMT	Homozygous variants (val/val and met/met) interact with trauma to predict PTSD.	Met/met carriers show poorer fear extinction learning compared to val carriers.
- 5-HTTLPR	s-allele interacts with trauma to promote increased PTSD symptoms.	s-allele associated with a stronger conditioned fear association that may be resistant to extinction.
- FKBP5	Shows exclusive interactions with early childhood trauma to predict PTSD.	Dexamethasone administration enhances extinction learning and retention via dynamic FKBP5 regulation in the HPA axis.
- PACAP	ADCYAP1R1 SNP (rs2267735) interacts with high trauma load to predict PTSD.	PACAP-38 administration results in impaired fear extinction recall in rodents.
Hormones		
- Cortisol	Cross-sectional research suggests reduced naturally cycling cortisol levels in PTSD, and as a marker for treatment-resistant PTSD. Low peri-trauma cortisol predicts increased PTSD symptoms.	Stress-induced cortisol increases associated with reduced fear expression in an extinction context and an acquisition context. Greater cortisol levels tend to be associated with more effective fear extinction learning. Oral cortisol consumption reduced spider fear following exposure therapy.
- Noradrenaline	Increased noradrenaline associated with greater PTSD symptoms and frequency of intrusive memories. Lower plasma noradrenaline levels predict PTSD symptoms 5-months post-hospitalization.	Enhanced noradrenergic signaling enhances memory formation; not limited to the formation of conditioning or extinction memories.

- Estrogen	Luteal phase (high estrogen) at time of trauma predicts greater PTSD symptoms, likely due to elevated glucocorticoid signaling during this phase of the menstrual cycle.	Follicular phase (low estrogen) associated with poorer fear extinction recall. Suggested that high estrogen enhances memory consolidation
Cognitive factors	PTSD associated with significant deficits in intellectual functioning, attention, learning, and memory. In particular, PTSD is most commonly associated with impairments in verbal learning and memory.	Poorer fear extinction learning ability while concurrently performing a verbal task, or under situations of greater cognitive load. Lower verbal learning and memory ability predicts poor responding to eight sessions of cognitive behavioral therapy.
Sleep disturbances	Sleep disturbances at the time of trauma significantly predict increased PTSD symptoms. Greater daytime sleepiness and insomnia post-trauma predicts increased PTSD symptoms.	Greater sleep deprivation associated with poorer fear extinction learning. Extinction learning ability best soon after waking, and declines throughout the day. Improved sleep quality enhances fear extinction learning and recall, and there is evidence for the reverse, that fear extinction learning improves sleep quality.

2.10.1 Theoretical implications.

The current review highlights the need to establish a timeline or sequence of biological and environmental events that lead to the development of PTSD following a traumatic stressor. The development of such a timeline allows for the integration of multiple

theories of PTSD development. For example, certain genotypes affect the regulation of the neuroendocrine system, and the negative feedback loop of the HPA axis, in some cases, via epigenetic interactions. These processes impair general fear extinction learning and retention ability. The occurrence of a traumatic event results in the acquisition of fear that is resistant to extinction via dysregulation of the HPA axis and neuroendocrine system, with contributions from the vmPFC, hippocampus and amygdala. Additionally, the occurrence of a new traumatic event may interact with the psychological consequences of previous trauma history to promote severe responding. Following trauma, fear extinction is hindered by the development of negative appraisals about the self and the trauma that exacerbate symptoms, and nightmares and sleep disturbances further prevent recovery. This is one of many possibilities by which a biological profile may confer risk for PTSD, and does not take into account the complexities of gene × environment interactions, or the countless influences of the environment (i.e., social support). Nevertheless, this review highlights an important need to understand the sequential "chain of events" that might lead to increased PTSD symptomatology.

The present review also carries important implications for well-established and empirically tested models of PTSD. One such theory is the cognitive model of PTSD (Ehlers & Clark, 2000), which proposed that PTSD symptoms persist as a result of negative appraisals of the self and the trauma, and due to fragmented and poorly elaborated autobiographical memories of the trauma. The avoidance of trauma reminders further exacerbates symptoms and affirms these negative appraisals. Fear extinction may be an important mechanism of this theory, with negative appraisals of the trauma and its sequelae providing an attentional bias to trauma reminders in the environment. These environmental and cognitive triggers result in intrusive and distressing memories of the trauma, which can be thought of as conditioned emotional/fear responses. These conditioned responses persist

as a result of impaired extinction, and avoidance of these trauma reminders further facilitate ongoing fear responding, and hindering extinction. While these two well-respected and well-supported models (i.e., biological fear extinction, and Ehlers and Clark's cognitive model) are not mutually exclusive, little research has been conducted to examine the convergent aspects of these models.

2.10.2 Clinical implications.

Should the ideas set forth in this review hold true, this model carries significant implications for various clinical and applied settings. Specifically, further understanding these qualities may allow for interventions to boost extinction learning and consolidation, thereby increasing resilience to negative reactions following a trauma. This scenario could lead to the redirection of clinical resources to early interventions pre- and post-trauma to prevent PTSD onset, thereby reducing negative coping in the aftermath of trauma and increasing quality of life.

A recent review discusses important pharmacological evidence that fear extinction can be enhanced via manipulation of key neurochemical imbalances and the promotion of synaptic plasticity in neural regions involved in the fear response and associative learning (Singewald et al., 2015). Singewald et al. (2015) discuss the effects of manipulating some of the factors discussed in the current review, such as BDNF, serotonin, and noradrenaline. For example, in section 2.2.2 (noradrenaline) of the current review, we discuss the research that enhanced pharmacological activation of noradrenergic signaling resulted in more efficient retrieval of extinction learning (e.g., Mueller et al., 2008). Pharmacological agents show significant promise in boosting fear extinction learning ability and the retention of extinction (for a comprehensive review, see Singewald et al., 2015). Importantly, the combination of

pharmacological intervention with exposure-based therapies may enhance fear extinction and prevent the return of fear.

The present review highlights the centrality of fear extinction memory in PTSD, and extinction as a possible mechanism linking risk factors to PTSD. At current, exposure-based treatments are among the most common treatment options for PTSD, based on the principles of extinction training to trauma-relevant stimuli. This review poses additional factors to be taken into account during treatment. Research suggests that fear extinction learning and memory are malleable constructs, with the potential for change. Time-of-day has revealed extinction learning, recall, and the generalization of extinction recall are significantly better in the morning compared to the evening in healthy participants (Pace-Schott et al., 2013). Similarly, healthy participants instructed with a verbal worrying task showed impaired fear extinction learning (as indexed by SCR and US-expectancy) compared to control groups (Gazendam & Kindt, 2012). Further, biological research has revealed that healthy women in the mid-follicular phase of the menstrual cycle display significantly poorer extinction memory compared to early-follicular healthy women and men (Milad, Goldstein, et al., 2006). To summarise, studies have identified that, in healthy populations, fear extinction memory can be altered as a function of time-of-day, cognitive resources devoted to a verbal task, and menstrual phase in women. While the target of exposure-based treatments is to extinguish the conditioned fear trace of the trauma memory, the above findings suggest that when treatment occurs, other factors should be taken into account. Indeed, our lab recently identified that extinction learning ability is significantly worse in participants with PTSD as they are awake for longer (Zuj et al., 2016), supporting the idea that exposure therapy would be more effective earlier in the day, rather than later (Pace-Schott et al., 2013). Further, extinction learning in women appears dynamic with menstrual phase (Milad, Goldstein, et al., 2006), and exposure therapy may be more effective during the luteal phase,

characterized by higher levels of estrogen, which is shown to be involved in enhanced memory consolidation.

2.10.3 Directions for future research.

Further research is required to explore the key links highlighted within this review. Should this conceptual model hold true, these findings carry important implications. In order to assess this extinction-based model of PTSD and other fear-related disorders, we suggest a number of research directions. First, longitudinal studies in first responder and military populations pre-trauma provide unique insights into the interplay between different factors (including fear extinction), and the role that each of these might play in the aftermath of trauma. In particular, the use of mediation and moderation analyses in such studies would allow the identification of causal relationships, as opposed to interactions between two or more variables to enhance PTSD risk. The findings of such designs would further our understanding of the temporal sequence of events that lead to PTSD development versus resilience. Currently, the temporal sequence beginning with trauma exposure and leading to increased PTSD symptoms is unknown, and improving our understanding of this sequence of events may greatly enhance the development of early interventions post-trauma to prevent the onset/persistence of symptoms.

Due to the expensive and time-consuming nature of prospective designs, cross-sectional research in PTSD compared to trauma non-exposed controls presents a unique opportunity to determine extinction learning and recall differences between trauma-exposed and non-exposed persons. Such designs that also include risk factors and biomarkers presented in the current review would increase our current understanding of PTSD risk, including the potential to shed light on certain inconsistencies (e.g., lower vs. higher cortisol levels conferring risk for PTSD). Third, a lack of genome-wide association studies

demonstrates a considerable gap in the literature of genetic and heritable biomarkers for PTSD and fear extinction potential. In the current review, we discuss the role of only a small selection of candidate genotypes and their role in PTSD and fear extinction. The identification of additional genetic risk factors would aid in further understanding the genetic makeup that predisposes an individual to severe posttraumatic stress.

In addition to the select risk factors presented in the current review, prior trauma history may also play a key role. For example, previous evidence indicates that female rape victims show significantly lower cortisol levels and greater risk of PTSD if they have experience prior assaults (Resnick, Yehuda, Pitman, & Foy, 1995). More recently, Najavits and Walsh (2012) found that high dissociation symptoms in PTSD were associated with a history of early life trauma (see also Evren et al., 2011; Schafer et al., 2010). With the important role that previous trauma can play in responding to a current traumatic event, it is likely that trauma history may impair the ability to extinguish fear associated with new traumatic events. For example, as a person experiences more traumatic events, their inherent ability to extinguish a fearful association is reduced, resulting in greater PTSD risk. To our knowledge, however, no studies have examined this.

In this review, we have highlighted the centrality of fear extinction learning and memory, with influences from a number of biological and cognitive factors that also serve as risk factors for PTSD. Further, we note that the sequential manner by which fear extinction affects, or is affected by these biological and cognitive factors is unknown. Future research using moderation and mediation models may prove useful in identifying causal relationships and important interactions involving fear extinction and PTSD symptoms. Studies such as these carry important implications for treatment, and additional studies using mediation and moderation models may identify further variables that could aid or impair treatment outcome (e.g., stress and sex hormone levels at the time of treatment).

2.10.4 Final comment.

In the present review, we discuss many of the risk factors for developing PTSD and their influence on fear extinction, carrying important implications for the centrality of fear extinction in PTSD. This notion has important clinical implications, with these risk factors potentially affecting exposure-based treatments of PTSD. However the influence of many of these variables on exposure therapy outcome in PTSD is yet to be explored, and may aid in improving current treatment models, and in improving treatment itself. In short, research is consistent that risk factors of PTSD share an important relationship with fear extinction memory, and we suggest that fear extinction may be a central variable linking these risk factors to PTSD development and symptom persistence.

2.11 References

Abercrombie, H. C., Kalin, N. H., Thurow, M. E., Rosenkranz, M. A., & Davidson, R. J. (2003). Cortisol variation in humans affects memory for emotionally laden and neutral information. *Behavioral Neuroscience, 117*(3), 505-516.

Adhikari, A., Lerner, T. N., Finkelstein, J., Pak, S., Jennings, J. H., Davidson, T. J., . . . Deisseroth, K. (2015). Basomedial amygdala mediates top-down control of anxiety and fear. *Nature, 527*(7577), 179-185. doi:10.1038/nature15698

Almli, L. M., Mercer, K. B., Kerley, K., Feng, H., Bradley, B., Conneely, K. N., & Ressler, K. J. (2013). ADCYAP1R1 genotype associates with post-traumatic stress symptoms in highly traumatized African-American females. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics, 162b*(3), 262-272. doi:10.1002/ajmg.b.32145

American Psychiatric Association. (2000). *Diagnostic and Statistical Manual of Mental Disorders* (4th ed., text revision ed.). Washington, DC: American Psychiatric Association.

American Psychiatric Association. (2013). *Diagnostic and Statistical Manual of Mental Disorders* (5th ed.). Washington, DC: Author.

Angelucci, F., Ricci, V., Gelfo, F., Martinotti, G., Brunetti, M., Sepede, G., . . . Caltagirone, C. (2014). BDNF serum levels in subjects developing or not post-traumatic stress disorder after trauma exposure. *Brain and Cognition, 84*(1), 118-122. doi:10.1016/j.bandc.2013.11.012

Armstrong, T., Bilsky, S. A., Zhao, M., & Olatunji, B. O. (2013). Dwelling on potential threat cues: an eye movement marker for combat-related PTSD. *Depression and Anxiety, 30*(5), 497-502. doi:10.1002/da.22115

Barrett, D. H., Green, M. L., Morris, R., Giles, W. H., & Croft, J. B. (1996). Cognitive functioning and posttraumatic stress disorder. *American Journal of Psychiatry, 153*(11), 1492-1494.

Beers, S. R., & De Bellis, M. D. (2002). Neuropsychological function in children with maltreatment-related posttraumatic stress disorder. *American Journal of Psychiatry, 159*(3), 483-486. doi:10.1176/appi.ajp.159.3.483

Begliuomini, S., Casarosa, E., Pluchino, N., Lenzi, E., Centofanti, M., Freschi, L., . . . Genazzani, A. R. (2007). Influence of endogenous and exogenous sex hormones on plasma brain-derived neurotrophic factor. *Human Reproduction, 22*(4), 995-1002. doi:10.1093/humrep/del479

Begliuomini, S., Lenzi, E., Ninni, F., Casarosa, E., Merlini, S., Pluchino, N., . . . Genazzani, A. R. (2008). Plasma brain-derived neurotrophic factor daily variations in men: correlation with cortisol circadian rhythm. *Journal of Endocrinology, 197*(2), 429-435. doi:10.1677/joe-07-0376

Bentz, D., Michael, T., Wilhelm, F. H., Hartmann, F. R., Kunz, S., von Rohr, I. R. R., & de Quervain, D. J. F. (2013). Influence of stress on fear memory processes in an aversive differential conditioning paradigm in humans. *Psychoneuroendocrinology, 38*(7), 1186-1197. doi:10.1016/j.psyneuen.2012.12.018

Berger, W., Mehra, A., Lenoci, M., Metzler, T. J., Otte, C., Tarasovsky, G., . . . Neylan, T. C. (2010). Serum brain-derived neurotrophic factor predicts responses to escitalopram in chronic posttraumatic stress disorder. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 34*(7), 1279-1284. doi:10.1016/j.pnpbp.2010.07.008

Berlau, D. J., & McGaugh, J. L. (2006). Enhancement of extinction memory consolidation: the role of the noradrenergic and GABAergic systems within the basolateral amygdala. *Neurobiology of Learning and Memory, 86*(2), 123-132. doi:10.1016/j.nlm.2005.12.008

Bicanic, I. A., Postma, R. M., Sinnema, G., De Roos, C., Olff, M., Van Wesel, F., & Van de Putte, E. M. (2013). Salivary cortisol and dehydroepiandrosterone sulfate in adolescent rape victims with post traumatic stress disorder. *Psychoneuroendocrinology, 38*(3), 408-415. doi:10.1016/j.psyneuen.2012.06.015

Binder, E. B., Bradley, R. G., Liu, W., Epstein, M. P., Deveau, T. C., Mercer, K. B., . . . Ressler, K. J. (2008). Association of FKBP5 polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults. *Journal of the American Medical Association, 299*(11), 1291-1305. doi:10.1001/jama.299.11.1291

Blechert, J., Michael, T., Vriends, N., Margraf, J., & Wilhelm, F. H. (2007). Fear conditioning in posttraumatic stress disorder: Evidence for delayed extinction of autonomic, experiential, and behavioural responses. *Behaviour Research and Therapy, 45*(9), 2019-2033. doi:10.1016/j.brat.2007.02.012

Blechert, J., Michael, T., Williams, S. L., Purkis, H. M., & Wilhelm, F. H. (2008). When two paradigms meet: Does evaluative learning extinguish in differential fear conditioning? *Learning and Motivation, 39*(1), 58-70. doi:10.1016/j.lmot.2007.03.003

Boddez, Y., Baeyens, F., Luyten, L., Vansteenwegen, D., Hermans, D., & Beckers, T. (2013). Rating data are underrated: validity of US expectancy in human fear conditioning. *Journal of Behavior Therapy and Experimental Psychiatry, 44*(2), 201-206. doi:10.1016/j.jbtep.2012.08.003

Bomyea, J., Risbrough, V., & Lang, A. J. (2012). A consideration of select pre-trauma factors as key vulnerabilities in PTSD. *Clinical Psychology Review, 32*(7), 630-641. doi:10.1016/j.cpr.2012.06.008

Bos, M. G., Beckers, T., & Kindt, M. (2012). The effects of noradrenergic blockade on extinction in humans. *Biological Psychology, 89*(3), 598-605. doi:10.1016/j.biopsycho.2012.01.007

Bos, M. G., Beckers, T., & Kindt, M. (2014). Noradrenergic blockade of memory reconsolidation: a failure to reduce conditioned fear responding. *Frontiers in Behavioral Neuroscience, 8*, 412. doi:10.3389/fnbeh.2014.00412

Boscarino, J. A., Hoffman, S. N., Rukstalis, M., & Stewart, W. F. (2011). Association of FKBP5, COMT and CHRNA5 polymorphisms with PTSD among outpatients at risk for PTSD. *Psychiatry Research, 188*(1), 173-174. doi:10.1016/j.psychres.2011.03.002

Bremner, J. D., Vermetten, E., Afzal, N., & Vythilingam, M. (2004). Deficits in verbal declarative memory function in women with childhood sexual abuse-related

posttraumatic stress disorder. *Journal of Nervous and Mental Disease, 192*(10), 643-649. doi:10.1097/01.nmd.0000142027.52893.c8

Bremner, J. D., Vermetten, E., Schmahl, C., Vaccarino, V., Vythilingam, M., Afzal, N., . . . Charney, D. S. (2005). Positron emission tomographic imaging of neural correlates of a fear acquisition and extinction paradigm in women with childhood sexual-abuse-related post-traumatic stress disorder. *Psychological Medicine, 35*(6), 791-806. doi:10.1017/s0033291704003290

Breslau, N., Chen, Q. L., & Luo, Z. H. (2013). The role of intelligence in posttraumatic stress disorder: Does it vary by trauma severity? *PLoS ONE, 8*(6). doi:10.1371/journal.pone.0065391

Breslau, N., Roth, T., Burduvali, E., Kapke, A., Schultz, L., & Roehrs, T. (2004). Sleep in lifetime posttraumatic stress disorder: A community-based polysomnographic study. *Archives of General Psychiatry, 61*(5), 508-516. doi:10.1001/archpsyc.61.5.508

Brewin, C. R., Kleiner, J. S., Vasterling, J. J., & Field, A. P. (2007). Memory for emotionally neutral information in posttraumatic stress disorder: A meta-analytic investigation. *Journal of Abnormal Psychology, 116*(3), 448-463. doi:10.1037/0021-843x.116.3.448

Briscione, M. A., Jovanovic, T., & Norrholm, S. D. (2014). Conditioned fear associated phenotypes as robust, translational indices of trauma-, stressor-, and anxiety-related behaviors. *Frontiers in Psychiatry, 5*, 88. doi:10.3389/fpsyt.2014.00088

Brunet, A., Poundja, J., Tremblay, J., Bui, E., Thomas, E., Orr, S. P., . . . Pitman, R. K. (2011). Trauma reactivation under the influence of propranolol decreases posttraumatic stress symptoms and disorder: 3 open-label trials. *Journal of Clinical Psychopharmacology, 31*(4), 547-550. doi:10.1097/JCP.0b013e318222f360

Bryant, R. A. (2003). Early predictors of posttraumatic stress disorder. *Biological Psychiatry, 53*(9), 789-795. doi:10.1016/S0006-3223(02)01895-4

Bryant, R. A. (2011). Acute stress disorder as a predictor of posttraumatic stress disorder: A systematic review. *Journal of Clinical Psychiatry, 72*(2), 233-239. doi:10.4088/JCP.09r05072blu

Bryant, R. A., Creamer, M., O'Donnell, M., Silove, D., & McFarlane, A. C. (2010). Sleep disturbance immediately prior to trauma predicts subsequent psychiatric disorder. *Sleep, 33*(1), 69-74.

Bryant, R. A., Felmingham, K. L., Silove, D., Creamer, M., O'Donnell, M., & McFarlane, A. C. (2011). The association between menstrual cycle and traumatic memories. *Journal of Affective Disorders, 131*(1-3), 398-401. doi:10.1016/j.jad.2010.10.049

Buchel, C., Morris, J., Dolan, R. J., & Friston, K. J. (1998). Brain systems mediating aversive conditioning: An event-related fMRI study. *Neuron, 20*(5), 947-957. doi:10.1016/S0896-6273(00)80476-6

Burgos-Robles, A., Vidal-Gonzalez, I., & Quirk, G. J. (2009). Sustained conditioned responses in prelimbic prefrontal neurons are correlated with fear expression and extinction failure. *The Journal of Neuroscience, 29*(26), 8474-8482. doi:10.1523/jneurosci.0378-09.2009

Cahill, L. (2006). Why sex matters for neuroscience. *Nature Reviews Neuroscience, 7*(6), 477-484. doi:10.1038/nrn1909

Chang, S. C., Xie, P., Anton, R. F., De Vivo, I., Farrer, L. A., Kranzler, H. R., . . . Koenen, K. C. (2012). No association between ADCYAP1R1 and post-traumatic stress disorder in two independent samples. *Molecular Psychiatry, 17*(3), 239-241. doi:10.1038/mp.2011.118

Chen, J., Lipska, B. K., Halim, N., Ma, Q. D., Matsumoto, M., Melhem, S., . . . Weinberger, D. R. (2004). Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. *American Journal of Human Genetics, 75*(5), 807-821. doi:10.1086/425589

Cheng, D. T., Knight, D. C., Smith, C. N., Stein, E. A., & Helmstetter, F. J. (2003). Functional MRI of human amygdala activity during Pavlovian fear conditioning: Stimulus processing versus response expression. *Behavioral Neuroscience, 117*(1), 3-10. doi:10.1037/0735-7044.117.1.3

Cheng, D. T., Richards, J., & Helmstetter, F. J. (2007). Activity in the human amygdala corresponds to early, rather than late period autonomic responses to a signal for shock. *Learning and Memory, 14*(7), 485-490. doi:10.1101/lm.632007

Cheung, J., Chervonsky, L., Felmingham, K. L., & Bryant, R. A. (2013). The role of estrogen in intrusive memories. *Neurobiology of Learning and Memory, 106*, 87-94. doi:10.1016/j.nlm.2013.07.005

Clark, R., DeYoung, C. G., Sponheim, S. R., Bender, T. L., Polusny, M. A., Erbes, C. R., & Arbisi, P. A. (2013). Predicting post-traumatic stress disorder in veterans: Interaction of traumatic load with COMT gene variation. *Journal of Psychiatric Research, 47*(12), 1849-1856. doi:10.1016/j.jpsychires.2013.08.013

Corcoran, K. A., & Maren, S. (2001). Hippocampal inactivation disrupts contextual retrieval of fear memory after extinction. *The Journal of Neuroscience, 21*(5), 1720-1726.

Corcoran, K. A., & Quirk, G. J. (2007). Activity in prelimbic cortex is necessary for the expression of learned, but not innate, fears. *The Journal of Neuroscience, 27*(4), 840-844. doi:10.1523/jneurosci.5327-06.2007

Crowell, T. A., Kieffer, K. M., Siders, C. A., & Vanderploeg, R. D. (2002). Neuropsychological Findings in Combat-Related Posttraumatic Stress Disorder. *The Clinical Neuropsychologist, 16*(3), 310-321. doi:10.1076/clin.16.3.310.13851

Datta, S., & O'Malley, M. W. (2013). Fear extinction memory consolidation requires potentiation of pontine-wave activity during REM sleep. *The Journal of Neuroscience, 33*(10), 4561-4569. doi:10.1523/jneurosci.5525-12.2013

Davis, M., & Myers, K. M. (2002). The role of glutamate and gamma-aminobutyric acid in fear extinction: clinical implications for exposure therapy. *Biological Psychiatry, 52*(10), 998-1007.

de Quervain, D. J., Bentz, D., Michael, T., Bolt, O. C., Wiederhold, B. K., Margraf, J., & Wilhelm, F. H. (2011). Glucocorticoids enhance extinction-based psychotherapy. *Proceedings of the National Academy of Science of the United States of America, 108*(16), 6621-6625. doi:10.1073/pnas.1018214108

Debiec, J., Bush, D. E., & LeDoux, J. E. (2011). Noradrenergic enhancement of reconsolidation in the amygdala impairs extinction of conditioned fear in rats: A

possible mechanism for the persistence of traumatic memories in PTSD. *Depression and Anxiety, 28*(3), 186-193. doi:10.1002/da.20803

Delahanty, D. L., Nugent, N. R., Christopher, N. C., & Walsh, M. (2005). Initial urinary epinephrine and cortisol levels predict acute PTSD symptoms in child trauma victims. *Psychoneuroendocrinology, 30*(2), 121-128. doi:10.1016/j.psyneuen.2004.06.004

Delahanty, D. L., Raimonde, A. J., & Spoonster, E. (2000). Initial posttraumatic urinary cortisol levels predict subsequent PTSD symptoms in motor vehicle accident victims. *Biological Psychiatry, 48*, 940-947.

Dell'Osso, L., Carmassi, C., Del Debbio, A., Dell'Osso, M. C., Bianchi, C., da Pozzo, E., . . . Piccinni, A. (2009). Brain-derived neurotrophic factor plasma levels in patients suffering from post-traumatic stress disorder. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 33*(5), 899-902. doi:10.1016/j.pnpbp.2009.04.018

Dias, B. G., & Ressler, K. J. (2013). PACAP and the PAC1 receptor in post-traumatic stress disorder. *Neuropsychopharmacology, 38*(1), 245-246. doi:10.1038/npp.2012.147

Ehlers, A., & Clark, D. M. (2000). A cognitive model of posttraumatic stress disorder. *Behaviour Research and Therapy, 38*, 319-345.

Engdahl, B. E., Eberly, R. E., Hurwitz, T. D., Mahowald, M. W., & Blake, J. (2000). Sleep in a community sample of elderly war veterans with and without posttraumatic stress disorder. *Biological Psychiatry, 47*(6), 520-525. doi:10.1016/s0006-3223(99)00201-2

Evren, C., Sar, V., Dalbudak, E., Cetin, R., Durkaya, M., Evren, B., & Celik, S. (2011). Lifetime PTSD and quality of life among alcohol-dependent men: impact of childhood emotional abuse and dissociation. *Psychiatry Research, 186*(1), 85-90. doi:10.1016/j.psychres.2010.07.004

Fani, N., Tone, E. B., Phifer, J., Norrholm, S. D., Bradley, B., Ressler, K. J., . . . Jovanovic, T. (2012). Attention bias toward threat is associated with exaggerated fear expression and impaired extinction in PTSD. *Psychological Medicine, 42*(3), 533-543. doi:10.1017/s0033291711001565

Felmingham, K. L., Dobson-Stone, C., Schofield, P. R., Quirk, G. J., & Bryant, R. A. (2013). The brain-derived neurotrophic factor Val66Met polymorphism predicts response to exposure therapy in posttraumatic stress disorder. *Biological Psychiatry, 73*(11), 1059-1063. doi:10.1016/j.biopsych.2012.10.033

Felmingham, K. L., Fong, W. C., & Bryant, R. A. (2012). The impact of progesterone on memory consolidation of threatening images in women. *Psychoneuroendocrinology*. doi:10.1016/j.psyneuen.2012.03.026

Ferree, N. K., Kamat, R., & Cahill, L. (2011). Influences of menstrual cycle position and sex hormone levels on spontaneous intrusive recollections following emotional stimuli. *Consciousness and Cognition, 20*(4), 1154-1162. doi:10.1016/j.concog.2011.02.003

Flaks, M. K., Malta, S. M., Almeida, P. P., Bueno, O. F. A., Pupo, M. C., Andreoli, S. B., . . . Bressan, R. A. (2014). Attentional and executive functions are differentially affected by post-traumatic stress disorder and trauma. *Journal of Psychiatric Research, 48*(1), 32-39. doi:10.1016/j.jpsychires.2013.10.009

Frielingsdorf, H., Bath, K. G., Soliman, F., Difede, J., Casey, B. J., & Lee, F. S. (2010). Variant brain-derived neurotrophic factor Val66Met endophenotypes: implications for posttraumatic stress disorder. *Annals of the New York Academy of Sciences, 1208*, 150-157. doi:10.1111/j.1749-6632.2010.05722.x

Fu, J., Li, P., Ouyang, X., Gu, C., Song, Z., Gao, J., . . . Hu, B. (2007). Rapid eye movement sleep deprivation selectively impairs recall of fear extinction in hippocampus-independent tasks in rats. *Neuroscience, 144*(4), 1186-1192. doi:10.1016/j.neuroscience.2006.10.050

Fuller, R. W., Snoddy, H. D., & Perry, K. W. (1978). Effect of prazosin on norepinephrine concentration and turnover in rat brain and heart. *Archives Internationales de Pharmacodynamie et de Therapie, 231*(1), 30-41.

Gale, C. R., Deary, I. J., Boyle, S. H., Barefoot, J., Mortensen, L. H., & Batty, D. (2008). Cognitive ability in early adulthood and risk of 5 specific psychiatric disorders in middle age: The Vietnam experience study. *Archives of General Psychiatry, 65*(12), 1410-1418. doi:10.1001/archpsyc.65.12.1410

Gazendam, F. J., & Kindt, M. (2012). Worrying affects associative fear learning: A startle fear conditioning study. *PLoS ONE, 7*(4). doi:10.1371/journal.pone.0034882

George, S. A., Knox, D., Curtis, A. L., Aldridge, J. W., Valentino, R. J., & Liberzon, I. (2013). Altered locus coeruleus-norepinephrine function following single prolonged stress. *European Journal of Neuroscience, 37*(6), 901-909. doi:10.1111/ejn.12095

Geracioti, T. D., Jr., Baker, D. G., Ekhator, N. N., West, S. A., Hill, K. K., Bruce, A. B., . . . Kasckow, J. W. (2001). CSF norepinephrine concentrations in posttraumatic stress disorder. *The American Journal of Psychiatry, 158*(8), 1227-1230.

Germain, A. (2013). Sleep disturbances as the hallmark of PTSD: where are we now? *The American Journal of Psychiatry, 170*(4), 372-382. doi:10.1176/appi.ajp.2012.12040432

Germain, A., Buysse, D. J., & Nofzinger, E. (2008). Sleep-specific mechanisms underlying posttraumatic stress disorder: Integrative review and neurobiological hypotheses. *Sleep Medicine Reviews, 12*, 185-195. doi:10.1016/j.smrv.2007.09.003

Gilbertson, M. W., Gurvits, T. V., Lasko, N. B., Orr, S. P., & Pitman, R. K. (2001). Multivariate assessment of explicit memory function in combat veterans with posttraumatic stress disorder. *Journal of Traumatic Stress, 14*(2), 413-432. doi:10.1023/a:1011181305501

Gilbertson, M. W., Paulus, L. A., Williston, S. K., Gurvits, T. V., Lasko, N. B., Pitman, R. K., & Orr, S. P. (2006). Neurocognitive function in monozygotic twins discordant for combat exposure: Relationship to posttraumatic stress disorder. *Journal of Abnormal Psychology, 115*(3), 484-495. doi:10.1037/0021-843x.115-3.484

Glotzbach-Schoon, E., Andreatta, M., Reif, A., Ewald, H., Troeger, C., Baumann, C., . . . Pauli, P. (2013). Contextual fear conditioning in virtual reality is affected by 5HTTLPR and NPSR1 polymorphisms: effects on fear-potentiated startle. *Frontiers in Behavioral Neuroscience, 7*, 14. doi:10.3389/fnbeh.2013.00031

Glover, E. M., Jovanovic, T., Mercer, K. B., Kerley, K., Bradley, B., Ressler, K. J., & Norrholm, S. D. (2012). Estrogen levels are associated with extinction deficits in

women with posttraumatic stress disorder. *Biological Psychiatry, 72*(1), 19-24. doi:10.1016/j.biopsych.2012.02.031

Glover, E. M., Jovanovic, T., & Norrholm, S. D. (2015). Estrogen and extinction of fear memories: Implications for posttraumatic stress disorder treatment. *Biological Psychiatry, 78*(3), 178-185. doi:10.1016/j.biopsych.2015.02.007

Glover, E. M., Mercer, K. B., Norrholm, S. D., Davis, M., Duncan, E., Bradley, B., . . . Jovanovic, T. (2013). Inhibition of fear is differentially associated with cycling estrogen levels in women. *Journal of Psychiatry & Neuroscience, 38*(5), 341-348. doi:10.1503/jpn.120129

Graham, B. M., & Milad, M. R. (2013). Blockade of estrogen by hormonal contraceptives impairs fear extinction in female rats and women. *Biological Psychiatry, 73*(4), 371-378. doi:10.1016/j.biopsych.2012.09.018

Gressier, F., Calati, R., Balestri, M., Marsano, A., Alberti, S., Antypa, N., & Serretti, A. (2013). The 5-HTTLPR polymorphism and posttraumatic stress disorder: a meta-analysis. *Journal of Traumatic Stress, 26*(6), 645-653. doi:10.1002/jts.21855

Grigorovich, A., Gomez, M., Leach, L., & Fish, J. (2013). Impact of posttraumatic stress disorder and depression on neuropsychological functioning in electrical injury survivors. *Journal of Burn Care & Research, 34*(6), 659-665. doi:10.1097/BCR.0b013e31827e5062

Groer, M. W., Kane, B., Williams, S. N., & Duffy, A. (2014). Relationship of PTSD Symptoms With Combat Exposure, Stress, and Inflammation in American Soldiers. *Biological Research for Nursing*. doi:10.1177/1099800414544949

Guthrie, R. M., & Bryant, R. A. (2006). Extinction learning before trauma and subsequent posttraumatic stress. *Psychosomatic Medicine, 68*, 307-311. doi:10.1097/01.psy.0000208629.67653.cc

Gutner, C. A., Casement, M. D., Stavitsky Gilbert, K., & Resick, P. A. (2013). Change in sleep symptoms across Cognitive Processing Therapy and Prolonged Exposure: a longitudinal perspective. *Behaviour Research and Therapy, 51*(12), 817-822. doi:10.1016/j.brat.2013.09.008

Handa, R. J., Burgess, L. H., Kerr, J. E., & O'Keefe, J. A. (1994). Gonadal steroid hormone receptors and sex differences in the hypothalamo-pituitary-adrenal axis. *Hormones and Behavior, 28*(4), 464-476. doi:10.1006/hbeh.1994.1044

Hauck, S., Gomes, F., Silveira Junior Ede, M., Almeida, E., Possa, M., & Ceitlin, L. H. (2009). Serum levels of brain-derived neurotrophic factor in acute and posttraumatic stress disorder: a case report study. *Revista Brasileira de Psiquiatria, 31*(1), 48-51.

Hauck, S., Kapczinski, F., Roesler, R., Silveira, E. D., Magalhaes, P. V., Kruel, L. R. P., . . . Ceitlin, L. H. F. (2010). Serum brain-derived neurotrophic factor in patients with trauma psychopathology. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 34*(3), 459-462. doi:10.1016/j.pnpbp.2010.01.010

Hefner, K., Whittle, N., Juhasz, J., Norcross, M., Karlsson, R. M., Saksida, L. M., . . . Holmes, A. (2008). Impaired fear extinction learning and cortico-amygdala circuit abnormalities in a common genetic mouse strain. *The Journal of Neuroscience, 28*(32), 8074-8085. doi:10.1523/JNEUROSCI.4904-07.2008

Heitland, I., Groenink, L., Bijlsma, E. Y., Oosting, R. S., & Baas, J. M. P. (2013). Human fear acquisition deficits in relation to genetic variants of the corticotropin releasing hormone receptor 1 and the serotonin transporter. *PLoS ONE, 8*(5), 10. doi:10.1371/journal.pone.0063772

Hettema, J. M., Annas, P., Neale, M. C., Kendler, K. S., & Fredrikson, M. (2003). A twin study of the genetics of fear conditioning. *Archives of General Psychiatry, 60*(7), 702-708. doi:10.1001/archpsyc.60.7.702

Holmes, A., & Singewald, N. (2013). Individual differences in recovery from traumatic fear. *Trends in Neurosciences, 36*(1), 23-31. doi:10.1016/j.tins.2012.11.003

Horner, M. D., Mintzer, J. E., Turner, T. H., Edmiston, K. R., & Brawman-Mintzer, O. (2013). Attentional functioning in patients with posttraumatic stress disorder: a preliminary study. *CNS Spectrums, 18*(2), 90-94. doi:10.1017/s1092852912000909

Hurwitz, T. D., Mahowald, M. W., Kuskowski, M., & Engdahl, B. E. (1998). Polysomnographic sleep is not clinically impaired in Vietnam combat veterans with chronic posttraumatic stress disorder. *Biological Psychiatry, 44*(10), 1066-1073. doi:10.1016/s0006-3223(98)00089-4

Jelinek, L., Jacobsen, D., Kellner, M., Larbig, F., Biesold, K. H., Barre, K., & Moritz, S. (2006). Verbal and nonverbal memory functioning in posttraumatic stress disorder (PTSD). *Journal of Clinical and Experimental Neuropsychology, 28*(6), 940-948. doi:10.1080/13803390591004347

Johnson, L. R., McGuire, J., Lazarus, R., & Palmer, A. A. (2012). Pavlovian fear memory circuits and phenotype models of PTSD. *Neuropharmacology, 62*(2), 638-646. doi:10.1016/j.neuropharm.2011.07.004

Jovanovic, T., Norrholm, S. D., Blanding, N. Q., Davis, M., Duncan, E., Bradley, B., & Ressler, K. J. (2010). Impaired fear inhibition is a biomarker of PTSD but not depression. *Depression and Anxiety, 27*(3), 244-251. doi:10.1002/da.20663

Jovanovic, T., Norrholm, S. D., Fennell, J. E., Keyes, M., Fiallos, A. M., Myers, K. M., . . . Duncan, E. J. (2009). Posttraumatic stress disorder may be associated with impaired fear inhibition: Relation to symptom severity. *Psychiatry Research, 167*(1-2), 151-160. doi:10.1016/j.psychres.2007.12.014

Kessler, R. C., Chiu, W. T., Demler, O., Merikangas, K. R., & Walters, E. E. (2005). Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. *Archives of General Psychiatry, 62*(6), 617-627. doi:10.1001/archpsyc.62.6.617

Kessler, R. C., Sonnega, A., Bromet, E., Hughes, M., & Nelson, C. B. (1995). Posttraumatic stress disorder in the National Comorbidity Survey. *Archives of General Psychiatry, 52*(12), 1048-1060.

Kilpatrick, D. G., Koenen, K. C., Ruggiero, K. J., Acierno, R., Galea, S., Resnick, H. S., . . . Gelernter, J. (2007). The serotonin transporter genotype and social support and moderation of posttraumatic stress disorder and depression in hurricane-exposed adults. *American Journal of Psychiatry, 164*(11), 1693-1699. doi:10.1176/appi.ajp.2007.06122007

Kindt, M., Soeter, M., & Sevenster, D. (2014). Disrupting reconsolidation of fear memory in humans by a noradrenergic beta-blocker. *Journal of Visualized Experiments*(94). doi:10.3791/52151

Kleim, B., Wilhelm, F. H., Temp, L., Margraf, J., Wiederhold, B. K., & Rasch, B. (2014). Sleep enhances exposure therapy. *Psychological Medicine*, 1-9. doi:10.1017/s0033291713001748

Knapska, E., & Maren, S. (2009). Reciprocal patterns of c-Fos expression in the medial prefrontal cortex and amygdala after extinction and renewal of conditioned fear. *Learning and Memory, 16*(8), 486-493. doi:10.1101/lm.1463909

Knight, D. C., Smith, C. N., Cheng, D. T., Stein, E. A., & Helmstetter, F. J. (2004). Amygdala and hippocampal activity during acquisition and extinction of human fear conditioning. *Cognitive, Affective, & Behavioral Neuroscience, 4*(3), 317-325.

Kobayashi, I., Boarts, J. M., & Delahanty, D. L. (2007). Polysomnographically measured sleep abnormalities in PTSD: A meta-analytic review. *Psychophysiology, 44*, 660-669. doi:10.1111/j.1469-8986.2007.537

Koenen, K. C., Aiello, A. E., Bakshis, E., Amstadter, A. B., Ruggiero, K. J., Acierno, R., . . . Galea, S. (2009). Modification of the association between serotonin transporter genotype and risk of posttraumatic stress disorder in adults by county-level social environment. *American Journal of Epidemiology, 169*(6), 704-711. doi:10.1093/aje/kwn397

Kolassa, I.-T., Kolassa, S., Ertl, V., Papassotiropoulos, A., & De Quervain, D. J. F. (2010). The risk of posttraumatic stress disorder after trauma depends on traumatic load and the Catechol-O-Methyltransferase Val158Met polymorphism. *Biological Psychiatry, 67*(4), 304-308. doi:10.1016/j.biopsych.2009.10.009

Kolb, L. C., & Mutalipassi, L. R. (1982). The conditioned emotional response: A sub-class of the chronic and delayed post-traumatic stress disorder. *Psychiatric Annals, 12*(11), 979-987.

Koren, D., Arnon, I., Lavie, P., & Klein, E. (2002). Sleep complaints as early predictors of posttraumatic stress disorder: A 1-year prospective study of injured survivors of motor vehicle accidents. *The American Journal of Psychiatry, 159*, 855-857.

Kosten, T. R., Mason, J. W., Giller, E. L., Ostroff, R. B., & Harkness, L. (1987). Sustained urinary norepinephrine and epinephrine elevation in post-traumatic stress disorder. *Psychoneuroendocrinology, 12*(1), 13-20.

Krakow, B., Hollifield, M., Johnston, L., Koss, M., Schrader, R., Warner, T. D., . . . Prince, H. (2001). Imagery rehearsal therapy for chronic nightmares in sexual assault survivors with posttraumatic stress disorder: A randomized controlled trial. *Journal of the American Medical Association, 286*(5), 537-545. doi:10.1001/jama.286.5.537

Kremen, W. S., Koenen, K. C., Afari, N., & Lyons, M. J. (2012). Twin studies of posttraumatic stress disorder: Differentiating vulnerability factors from sequelae. *Neuropharmacology, 62*, 647-653. doi:10.1016/j.neuropharm.2011.03.012

Kremen, W. S., Koenen, K. C., Boake, C., Purcell, S., Eisen, S. A., Franz, C. E., . . . Lyons, M. J. (2007). Pretrauma cognitive ability and risk for posttraumatic stress disorder - A twin study. *Archives of General Psychiatry, 64*(3), 361-368. doi:10.1001/archpsyc.64.3.361

LaBar, K. S., Gatenby, J. C., Gore, J. C., LeDoux, J. E., & Phelps, E. A. (1998). Human amygdala activation during conditioned fear acquisition and extinction: a mixed-trial fMRI study. *Neuron, 20*(5), 937-945.

Lang, S., Kroll, A., Lipinski, S. J., Wessa, M., Ridder, S., Christmann, C., . . . Flor, H. (2009). Context conditioning and extinction in humans: differential contribution of the hippocampus, amygdala and prefrontal cortex. *European Journal of Neuroscience, 29*(4), 823-832. doi:10.1111/j.1460-9568.2009.06624.x

Laurent, V., & Westbrook, R. F. (2009). Inactivation of the infralimbic but not the prelimbic cortex impairs consolidation and retrieval of fear extinction. *Learning and Memory, 16*(9), 520-529. doi:10.1101/lm.1474609

Lebron-Milad, K., & Milad, M. R. (2012). Sex differences, gonadal hormones and the fear extinction network: implications for anxiety disorders. *Biology of Mood & Anxiety Disorders, 2*. doi:10.1186/2045-5380-2-3

LeDoux, J. E. (2000). Emotion circuits in the brain. *Annual Review of Neuroscience, 23*, 155-184. doi:10.1146/annurev.neuro.23.1.155

Lee, H. J., Lee, M. S., Kang, R. H., Kim, H., Yim, S. D., Kee, B. S., . . . Paik, I. H. (2005). Influence of the serotonin transporter promoter gene polymorphism on susceptibility to posttraumatic stress disorder. *Depression and Anxiety, 21*(3), 135-139. doi:10.1002/da.20064

Lesch, K. P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S., . . . Murphy, D. L. (1996). Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science, 274*(5292), 1527-1531.

Levin, R., & Nielsen, T. A. (2007). Disturbed dreaming, posttraumatic stress disorder, and affect distress: a review and neurocognitive model. *Psychological Bulletin, 133*(3), 482-528. doi:10.1037/0033-2909.133.3.482

Levy-Gigi, E., Szabo, C., Kelemen, O., & Keri, S. (2013). Association among clinical response, hippocampal volume, and FKBP5 gene expression in individuals with posttraumatic stress disorder receiving cognitive behavioral therapy. *Biological Psychiatry, 74*(11), 793-800. doi:10.1016/j.biopsych.2013.05.017

Linnman, C., Rougemont-Bucking, A., Beucke, J. C., Zeffiro, T. A., & Milad, M. R. (2011). Unconditioned responses and functional fear networks in human classical conditioning. *Behavioural Brain Research, 221*(1), 237-245. doi:10.1016/j.bbr.2011.02.045

Linnman, C., Zeffiro, T. A., Pitman, R. K., & Milad, M. R. (2011). An fMRI study of unconditioned responses in post-traumatic stress disorder. *Biology of Mood & Anxiety Disorders, 1*(1), 8. doi:10.1186/2045-5380-1-8

Lommen, M. J. J., Engelhard, I. M., Sijbrandij, M., van den Hout, M. A., & Hermans, D. (2013). Pre-trauma individual differences in extinction learning predict posttraumatic stress. *Behaviour Research and Therapy, 51*(2), 63-67. doi:10.1016/j.brat.2012.11.004

Lommen, M. J. J., Grey, N., Clark, D. M., Wild, J., Stott, R., & Ehlers, A. (2016). Sleep and treatment outcome in posttraumatic stress disorder: Results from an effectiveness study. *Depression and Anxiety, 33*(7), 575-583. doi:10.1002/da.22420

Long, M. E., Hammons, M. E., Davis, J. L., Frueh, B. C., Khan, M. M., Elhai, J. D., & Teng, E. J. (2011). Imagery rescripting and exposure group treatment of posttraumatic nightmares in Veterans with PTSD. *Journal of Anxiety Disorders, 25*(4), 531-535. doi:10.1016/j.janxdis.2010.12.007

Lonsdorf, T. B., Golkar, A., Lindstrom, K. M., Haaker, J., Ohman, A., Schalling, M., & Ingvar, M. (2015). BDNFval66met affects neural activation pattern during fear conditioning and 24 h delayed fear recall. *Social Cogntiive & Affective Neuroscience, 10*(5), 664-671. doi:10.1093/scan/nsu102

Lonsdorf, T. B., Ruck, C., Bergstrom, J., Andersson, G., Ohman, A., Lindefors, N., & Schalling, M. (2010). The COMTval158met polymorphism is associated with symptom relief during exposure-based cognitive-behavioral treatment in panic disorder. *BMC Psychiatry, 10*. doi:10.1186/1471-244x-10-99

Lonsdorf, T. B., Weike, A. I., Nikamo, P., Schalling, M., Hamm, A. O., & Ohman, A. (2009). Genetic gating of human fear learning and extinction: Possible implications for gene-environment interaction in anxiety disorder. *Psychological Science, 20*(2), 198-206. doi:10.1111/j.1467-9280.2009.02280.x

Macklin, M. L., Metzger, L. J., Litz, B. T., McNally, R. J., Lasko, N. B., Orr, S. P., & Pitman, R. K. (1998). Lower precombat intelligence is a risk factor for posttraumatic stress disorder. *Journal of Consulting and Clinical Psychology, 66*(2), 323-326.

Maren, S., Phan, K. L., & Liberzon, I. (2013). The contextual brain: implications for fear conditioning, extinction and psychopathology. *Nature Reviews Neuroscience, 14*(6), 417-428. doi:10.1038/nrn3492

Marx, B. P., Brailey, K., Proctor, S. P., MacDonald, H. Z., Graefe, A. C., Amoroso, P., . . . Vasterling, J. J. (2009). Association of time since deployment, combat intensity, and posttraumatic stress symptoms with neuropsychological outcomes following Iraq war deployment. *Archives of General Psychiatry, 66*(9), 996-1004.

Marx, B. P., Doron-Lamarca, S., Proctor, S. P., & Vasterling, J. J. (2009). The influence of pre-deployment neurocognitive functioning on post-deployment PTSD symptom outcomes among Iraq-deployed Army soldiers. *Journal of the International Neuropsychological Society, 15*(6), 840-852. doi:10.1017/S1355617709990488

Matsuoka, Y., Nishi, D., Noguchi, H., Kim, Y., & Hashimoto, K. (2013). Longitudinal changes in serum brain-derived neurotrophic factor in accident survivors with posttraumatic stress disorder. *Neuropsychobiology, 68*(1), 44-50. doi:10.1159/000350950

McFarlane, A. C., Atchison, M., & Yehuda, R. (1997). The acute stress response following motor vehicle accidents and its relation to PTSD. *Annals of the New York Academy of Sciences, 821*, 437-441.

McFarlane, A. C., Barton, C. A., Yehuda, R., & Wittert, G. (2011). Cortisol response to acute trauma and risk of posttraumatic stress disorder. *Psychoneuroendocrinology, 36*(5), 720-727. doi:10.1016/j.psyneuen.2010.10.007

McGaugh, J. L. (2004). The amygdala modulates the consolidation of memories of emotionally arousing experiences. *Annual Review of Neuroscience, 27*, 1-28. doi:10.1146/annurev.neuro.27.070203.144157

McLean, C. P., Asnaani, A., Litz, B. T., & Hofmann, S. G. (2011). Gender differences in anxiety disorders: Prevalence, course of illness, comorbidity and burden of illness. *Journal of Psychiatric Research, 45*(8), 1027-1035. doi:10.1016/j.jpsychires.2011.03.006

McNally, R. J. (2006). Cognitive abnormalities in post-traumatic stress disorder. *Trends in Cognitive Sciences, 10*(6), 271-277. doi:10.1016/j.tics.2006.04.007

Medina, J. F., Repa, J. C., Mauk, M. D., & LeDoux, J. E. (2002). Parallels between cerebellum- and amygdala-dependent conditioning. *Nature Reviews Neuroscience, 3*(2), 122-131. doi:10.1038/nrn728

Mehta, D., Gonik, M., Klengel, T., Rex-Haffner, M., Menke, A., Rubel, J., . . . Binder, E. B. (2011). Using polymorphisms in FKBP5 to define biologically distinct subtypes of posttraumatic stress disorder evidence from endocrine and gene expression studies. *Archives of General Psychiatry, 68*(9), 901-910. doi:10.1001/archgenpsychiatry.2011.50

Mellman, T. A., Alim, T., Brown, D. D., Gorodetsky, E., Buzas, B., Lawson, W. B., . . . Charney, D. S. (2009). Serotonin polymorphisms and posttraumatic stress disorder in a trauma exposed African American population. *Depression and Anxiety, 26*(11), 993-997. doi:10.1002/da.20627

Mellman, T. A., Bustamante, V., Fins, A. I., Pigeon, W. R., & Nolan, B. (2002). REM sleep and the early development of posttraumatic stress disorder. *American Journal of Psychiatry, 159*(10), 1696-1701.

Mellman, T. A., & Hipolito, M. M. (2006). Sleep disturbances in the aftermath of trauma and posttraumatic stress disorder. *CNS Spectrums, 11*(8), 611-615.

Mellman, T. A., Knorr, B. R., Pigeon, W. R., Leiter, J. C., & Akay, M. (2004). Heart rate variability during sleep and the early development of posttraumatic stress disorder. *Biological Psychiatry, 55*(9), 953-956. doi:10.1016/j.biopsych.2003.12.018

Mellman, T. A., Kumar, A., Kulickbell, R., Kumar, M., & Nolan, B. (1995). Nocturnal/daytime urine noradrenergic measures and sleep in combat-related PTSD. *Biological Psychiatry, 38*(3), 174-179. doi:10.1016/0006-3223(94)00238-x

Mellman, T. A., Nolan, B., Hebding, J., KulickBell, R., & Dominguez, R. (1997). A polysomnographic comparison of veterans with combat-related PTSD, depressed men, and non-ill controls. *Sleep, 20*(1), 46-51.

Mellman, T. A., Pigeon, W. R., Nowell, P. D., & Nolan, B. (2007). Relationships between REM sleep findings and PTSD symptoms during the early aftermath of trauma. *Journal of Traumatic Stress, 20*(5), 893-901. doi:10.1002/jts.20246

Merz, C. J., Hamacher-Dang, T. C., & Wolf, O. T. (2014). Exposure to stress attenuates fear retrieval in healthy men. *Psychoneuroendocrinology, 41*, 89-96. doi:10.1016/j.psyneuen.2013.12.009

Milad, M. R., Goldstein, J. M., Orr, S. P., Wedig, M. M., Klibanski, A., Pitman, R. K., & Rauch, S. L. (2006). Fear conditioning and extinction: Influence of sex and menstrual cycle in healthy humans. *Behavioral Neuroscience, 120*(6), 1196-1203. doi:10.1037/0735-7044.120.5.1196

Milad, M. R., Igoe, S. A., Lebron-Milad, K., & Novales, J. E. (2009). Estrous cycle phase and gonadal hormones influence conditioned fear extinction. *Neuroscience, 164*(3), 887-895. doi:10.1016/j.neuroscience.2009.09.011

Milad, M. R., Orr, S. P., Lasko, N. B., Chang, Y., Rauch, S. L., & Pitman, R. K. (2008). Presence and acquired origin of reduced recall for fear extinction in PTSD: Results of a twin study. *Journal of Psychiatric Research, 42*(7), 515-520. doi:10.1016/j.jpsychires.2008.01.017

Milad, M. R., Pitman, R. K., Ellis, C. B., Gold, A. L., Shin, L. M., Lasko, N. B., . . . Rauch, S. L. (2009). Neurobiological basis of failure to recall extinction memory in posttraumatic stress disorder. *Biological Psychiatry, 66*(12), 1075-1082. doi:10.1016/j.biopsych.2009.06.026

Milad, M. R., & Quirk, G. J. (2002). Neurons in medial prefrontal cortex signal memory for fear extinction. *Nature, 420*(6911), 70-74. doi:10.1038/nature01138

Milad, M. R., & Quirk, G. J. (2012). Fear extinction as a model for translational neuroscience: Ten years of progress. In S. T. Fiske, D. L. Schacter, & S. E. Taylor (Eds.), *Annual Review of Psychology* (Vol. 63, pp. 129-151).

Milad, M. R., Quirk, G. J., Pitman, R. K., Orr, S. P., Fischl, B., & Rauch, S. L. (2007). A role for the human dorsal anterior cingulate cortex in fear expression. *Biological Psychiatry, 62*(10), 1191-1194. doi:10.1016/j.biopsych.2007.04.032

Milad, M. R., Rauch, S. L., Pitman, R. K., & Quirk, G. J. (2006). Fear extinction in rats: Implications for human brain imaging and anxiety disorders. *Biological Psychology, 73*(1), 61-71. doi:10.1016/j.biopsycho.2006.01.008

Milad, M. R., Vidal-Gonzalez, I., & Quirk, G. J. (2004). Electrical stimulation of medial prefrontal cortex reduces conditioned fear in a temporally specific manner. *Behavioural Brain Research, 118*(2), 389-394. doi:10.1037/0735-7044.118.2.389

Milad, M. R., Wright, C. I., Orr, S. P., Pitman, R. K., Quirk, G. J., & Rauch, S. L. (2007). Recall of fear extinction in humans activates the ventromedial prefrontal cortex and hippocampus in concert. *Biological Psychiatry, 62*(5), 446-454. doi:10.1016/j.biopsych.2006.10.011

Milad, M. R., Zeidan, M. A., Contero, A., Pitman, R. K., Klibanski, A., Rauch, S. L., & Goldstein, J. M. (2010). The influence of gonadal hormones on conditioned fear extinction in healthy humans. *Neuroscience, 168*(3), 652-658. doi:10.1016/j.neuroscience.2010.04.030

Mineka, S., & Oehlberg, K. (2008). The relevance of recent developments in classical conditioning to understanding the etiology and maintenance of anxiety disorders. *Acta Psychologica, 127*(3), 567-580. doi:10.1016/j.actpsy.2007.11.007

Morris, J. S., & Dolan, R. J. (2004). Dissociable amygdala and orbitofrontal responses during reversal fear conditioning. *Neuroimage, 22*(1), 372-380. doi:10.1016/j.neuroimage.2004.01.012

Motzkin, J. C., Philippi, C. L., Wolf, R. C., Baskaya, M. K., & Koenigs, M. (2014). Ventromedial prefrontal cortex is critical for the regulation of amygdala activity in humans. *Biological Psychiatry*. doi:10.1016/j.biopsych.2014.02.014

Mueller, D., & Cahill, S. P. (2010). Noradrenergic modulation of extinction learning and exposure therapy. *Behavioural Brain Research, 208*(1), 1-11. doi:10.1016/j.bbr.2009.11.025

Mueller, D., Porter, J. T., & Quirk, G. J. (2008). Noradrenergic signaling in infralimbic cortex increases cell excitability and strengthens memory for fear extinction. *The Journal of Neuroscience, 28*(2), 369-375. doi:10.1523/jneurosci.3248-07.2008

Munck, A., & Guyre, P. M. (1986). Glucocorticoid physiology, pharmacology and stress. *Advances in Experimental Medicine and Biology, 196*, 81-96.

Myers, K. M., & Davis, M. (2007). Mechanisms of fear extinction. *Molecular Psychiatry, 12*(2), 120-150. doi:10.1038/sj.mp.4001939

Naim, R., Wald, I., Lior, A., Pine, D. S., Fox, N. A., Sheppes, G., . . . Bar-Haim, Y. (2013). Perturbed threat monitoring following a traumatic event predicts risk for post-traumatic stress disorder. *Psychological Medicine*, 1-8. doi:10.1017/s0033291713002456

Najavits, L. M., & Walsh, M. (2012). Dissociation, PTSD, and substance abuse: an empirical study. *Journal of Trauma & Dissociation, 13*(1), 115-126. doi:10.1080/15299732.2011.608781

Neylan, T. C., Lenoci, M., Rothlind, J., Metzler, T. J., Schuff, N., Du, A. T., . . . Marmar, C. R. (2004). Attention, learning, and memory in posttraumatic stress disorder. *Journal of Traumatic Stress, 17*(1), 41-46. doi:10.1023/B:JOTS.0000014675.75686.ee

Nicholson, E. L., Bryant, R. A., & Felmingham, K. L. (2014). Interaction of noradrenaline and cortisol predicts negative intrusive memories in posttraumatic stress disorder. *Neurobiology of Learning and Memory, 112*, 204-211. doi:10.1016/j.nlm.2013.11.018

Norrholm, S. D., Glover, E. M., Stevens, J. S., Fani, N., Galatzer-Levy, I. R., Bradley, B., . . . Jovanovic, T. (2015). Fear load: The psychophysiological over-expression of fear as an intermediate phenotype associated with trauma reactions. *International Journal of Psychophysiology, 98*(2 Pt 2), 270-275. doi:10.1016/j.ijpsycho.2014.11.005

Norrholm, S. D., Jovanovic, T., Olin, I. W., Sands, L. A., Karapanou, I., Bradley, B., & Ressler, K. J. (2011). Fear extinction in traumatized civilians with posttraumatic stress disorder: Relation to symptom severity. *Biological Psychiatry, 69*(6), 556-563. doi:10.1016/j.biopsych.2010.09.013

Norrholm, S. D., Jovanovic, T., Smith, A. K., Binder, E., Klengel, T., Conneely, K., . . . Ressler, K. J. (2013). Differential genetic and epigenetic regulation of catechol-O-methyltransferase is associated with impaired fear inhibition in posttraumatic stress disorder. *Frontiers in Behavioral Neuroscience, 7*, 10. doi:10.3389/fnbeh.2013.00030

Notaras, M., Hill, R., & van den Buuse, M. (2015). The BDNF gene Val66Met polymorphism as a modifier of psychiatric disorder susceptibility: progress and controversy. *Molecular Psychiatry, 20*(8), 916-930. doi:10.1038/mp.2015.27

Olatunji, B. O., Armstrong, T., McHugo, M., & Zald, D. H. (2013). Heightened attentional capture by threat in veterans with PTSD. *Journal of Abnormal Psychology, 122*(2), 397-405. doi:10.1037/a0030440

Orr, S. P., Lasko, N. B., Macklin, M. L., Pineles, S. L., Chang, Y., & Pitman, R. K. (2012). Predicting post-trauma stress symptoms from pre-trauma psychophysiologic reactivity, personality traits and measures of psychopathology. *Biology of Mood & Anxiety Disorders, 2*(8).

Orr, S. P., Metzger, L. J., Lasko, N. B., Macklin, M. L., Peri, T., & Pitman, R. K. (2000). De novo conditioning in trauma-exposed individuals with and without posttraumatic stress disorder. *Journal of Abnormal Psychology, 109*(2), 290-298. doi:10.1037/0021-843x.109.2.290

Orsini, C. A., Kim, J. H., Knapska, E., & Maren, S. (2011). Hippocampal and prefrontal projections to the basal amygdala mediate contextual regulation of fear after extinction. *The Journal of Neuroscience, 31*(47), 17269-17277. doi:10.1523/jneurosci.4095-11.2011

Orsini, C. A., & Maren, S. (2012). Neural and cellular mechanisms of fear and extinction memory formation. *Neuroscience and Biobehavioral Reviews, 36*(7), 1773-1802. doi:10.1016/j.neubiorev.2011.12.014

Pace-Schott, E. F., Germain, A., & Milad, M. R. (2015a). Effects of sleep on memory for conditioned fear and fear extinction. *Psychological Bulletin*. doi:10.1037/bul0000014

Pace-Schott, E. F., Germain, A., & Milad, M. R. (2015b). Sleep and REM sleep disturbance in the pathophysiology of PTSD: the role of extinction memory. *Biology of Mood & Anxiety Disorders, 5*, 3. doi:10.1186/s13587-015-0018-9

Pace-Schott, E. F., Milad, M. R., Orr, S. P., Rauch, S. L., Stickgold, R., & Pitman, R. K. (2009). Sleep promotes generalization of extinction of conditioned fear. *Sleep, 32*(1), 19-26.

Pace-Schott, E. F., Spencer, R. M. C., Vijayakumar, S., Ahmed, N. A. K., Verga, P. W., Orr, S. P., . . . Milad, M. R. (2013). Extinction of conditioned fear is better learned and recalled in the morning than in the evening. *Journal of Psychiatric Research, 47*(11), 1776-1784. doi:10.1016/j.jpsychires.2013.07.027

Pace-Schott, E. F., Verga, P. W., Bennett, T. S., & Spencer, R. M. C. (2012). Sleep promotes consolidation and generalization of extinction learning in simulated exposure therapy for spider fear. *Journal of Psychiatric Research, 46*, 1036-1044. doi:10.1016/j.jpsychires.2012.04.015

Pape, H. C., & Pare, D. (2010). Plastic synaptic networks of the amygdala for the acquisition, expression, and extinction of conditioned fear. *Physiological Reviews, 90*(2), 419-463. doi:10.1152/physrev.00037.2009

Pare, D., & Duvarci, S. (2012). Amygdala microcircuits mediating fear expression and extinction. *Current Opinion in Neurobiology, 22*(4), 717-723. doi:10.1016/j.conb.2012.02.014

Pare, D., Quirk, G. J., & Ledoux, J. E. (2004). New vistas on amygdala networks in conditioned fear. *Journal of Neurophysiology, 92*(1), 1-9. doi:10.1152/jn.00153.2004

Parslow, R. A., & Jorm, A. F. (2007). Pretrauma and posttrauma neurocognitive functioning and PTSD symptoms in a community sample of young adults. *The American Journal of Psychiatry, 164*(3), 509-515.

Pavlov, I. P. (1927). *Conditioned reflexes: An investigation of the physiological activity of the cerebral cortex*. London: Oxford University Press.

Peri, T., Ben-Shakhar, G., Orr, S. P., & Shalev, A. Y. (2000). Psychophysiologic assessment of aversive conditioning in posttraumatic stress disorder. *Biological Psychiatry, 47*(6), 512-519. doi:10.1016/s0006-3223(99)00144-4

Peters, J., Dieppa-Perea, L. M., Melendez, L. M., & Quirk, G. J. (2010). Induction of fear extinction with hippocampal-infralimbic BDNF. *Science, 328*(5983), 1288-1290. doi:10.1126/science.1186909

Phelps, E. A., Delgado, M. R., Nearing, K. I., & LeDoux, J. E. (2004). Extinction learning in humans: Role of the amygdala and vmPFC. *Neuron, 43*(6), 897-905. doi:10.1016/j.neuron.2004.08.042

Pietrzak, R. H., Gallezot, J. D., Ding, Y. S., Henry, S., Potenza, M. N., Southwick, S. M., . . . Neumeister, A. (2013). Association of posttraumatic stress disorder with reduced in vivo norepinephrine transporter availability in the locus coeruleus. *JAMA Psychiatry, 70*(11), 1199-1205. doi:10.1001/jamapsychiatry.2013.399

Pitman, R. K. (2011). Will reconsolidation blockade offer a novel treatment for posttraumatic stress disorder? *Frontiers in Behavioral Neuroscience, 5*, 11. doi:10.3389/fnbeh.2011.00011

Pitman, R. K., Rasmusson, A. M., Koenen, K. C., Shin, L. M., Orr, S. P., Gilbertson, M. W., . . . Liberzon, I. (2012). Biological studies of post-traumatic stress disorder. *Nature, 13*, 769-787. doi:10.1038/nm3339

Pitman, R. K., Sanders, K. M., Zusman, R. M., Healy, A. R., Cheema, F., Lasko, N. B., . . . Orr, S. P. (2002). Pilot study of secondary prevention of posttraumatic stress disorder with propranolol. *Biological Psychiatry, 51*(2), 189-192.

Psotta, L., Lessmann, V., & Endres, T. (2013). Impaired fear extinction learning in adult heterozygous BDNF knock-out mice. *Neurobiology of Learning and Memory, 103*, 34-38. doi:10.1016/j.nlm.2013.03.003

Quirk, G. J., & Mueller, D. (2008). Neural mechanisms of extinction learning and retrieval. *Neuropsychopharmacology, 33*(1), 56-72. doi:10.1038/sj.npp.1301555

Quirk, G. J., Russo, G. K., Barron, J. L., & Lebron, K. (2000). The role of ventromedial prefrontal cortex in the recovery of extinguished fear. *The Journal of Neuroscience, 20*(16), 6225-6231.

Raes, A. K., De Raedt, R., Verschuere, B., & De Houwer, J. (2009). Failure to loose fear: The impact of cognitive load and trait anxiety on extinction. *Behaviour Research and Therapy, 47*(12), 1096-1101. doi:10.1016/j.brat.2009.08.002

Raio, C. M., Brignoni-Perez, E., Goldman, R., & Phelps, E. A. (2014). Acute stress impairs the retrieval of extinction memory in humans. *Neurobiology of Learning and Memory, 112*, 212-221. doi:10.1016/j.nlm.2014.01.015

Rakofsky, J. J., Ressler, K. J., & Dunlop, B. W. (2012). BDNF function as a potential mediator of bipolar disorder and post-traumatic stress disorder comorbidity. *Molecular Psychiatry, 17*, 22-35. doi:10.1038/mp.2011.121

Ramchand, R., Schell, T. L., Karney, B. R., Osilla, K. C., Burns, R. M., & Caldarone, L. B. (2010). Disparate prevalence estimates of PTSD among service members who served

in Iraq and Afghanistan: possible explanations. *Journal of Traumatic Stress, 23*(1), 59-68. doi:10.1002/jts.20486

Raskind, M. A., Peskind, E. R., Hoff, D. J., Hart, K. L., Holmes, H. A., Warren, D., . . . McFall, M. E. (2007). A parallel group placebo controlled study of prazosin for trauma nightmares and sleep disturbance in combat veterans with post-traumatic stress disorder. *Biological Psychiatry, 61*(8), 928-934. doi:10.1016/j.biopsych.2006.06.032

Raskind, M. A., Peskind, E. R., Kanter, E. D., Petrie, E. C., Radant, A., Thompson, C. E., . . . McFall, M. M. (2003). Reduction of nightmares and other PTSD symptoms in combat veterans by prazosin: a placebo-controlled study. *American Journal of Psychiatry, 160*(2), 371-373.

Resnick, H. S., Yehuda, R., Pitman, R. K., & Foy, D. W. (1995). Effect of previous trauma on acute plasma cortisol level following rape. *American Journal of Psychiatry, 152*(11), 1675-1677. doi:10.1176/ajp.152.11.1675

Ressler, K. J., Mercer, K. B., Bradley, B., Jovanovic, T., Mahan, A., Kerley, K., . . . May, V. (2011). Post-traumatic stress disorder is associated with PACAP and the PAC1 receptor. *Nature, 470*(7335), 492-497. doi:10.1038/nature09856

Risbrough, V., Ji, B., Hauger, R., & Zhou, X. (2014). Generation and characterization of humanized mice carrying COMT158 met/val alleles. *Neuropsychopharmacology, 39*(8), 1823-1832. doi:10.1038/npp.2014.29

Rosa, J., Myskiw, J. C., Furini, C. R., Sapiras, G. G., & Izquierdo, I. (2014). Fear extinction can be made state-dependent on peripheral epinephrine: Role of norepinephrine in the nucleus tractus solitarius. *Neurobiology of Learning and Memory, 113*, 55-61. doi:10.1016/j.nlm.2013.09.018

Rosas-Vidal, L. E., Do-Monte, F. H., Sotres-Bayon, F., & Quirk, G. J. (2014). Hippocampal-prefrontal BDNF and memory for fear extinction. *Neuropsychopharmacology*. doi:10.1038/npp.2014.64

Ross, R. J., Ball, W. A., Dinges, D. F., Kribbs, N. B., Morrison, A. R., Silver, S. M., & Mulvaney, F. D. (1994a). Motor dysfunction during sleep in posttraumatic stress disorder. *Sleep, 17*(8), 723-732.

Ross, R. J., Ball, W. A., Dinges, D. F., Kribbs, N. B., Morrison, A. R., Silver, S. M., & Mulvaney, F. D. (1994b). Rapid eye movement sleep disturbance in posttraumatic stress disorder. *Biological Psychiatry, 35*(3), 195-202. doi:10.1016/0006-3223(94)91152-5

Ross, R. J., Ball, W. A., Sullivan, K. A., & Caroff, S. N. (1989). Sleep disturbance as the hallmark of posttraumatic stress disorder. *American Journal of Psychiatry, 146*(6), 697-707.

Rothbaum, B. O., & Davis, M. (2003). Applying learning principles to the treatment of post-trauma reactions. *Annals of the New York Academy of Sciences, 1008*, 112-121. doi:10.1196/annals.1301.012

Rougemont-Bücking, A., Linnman, C., Zeffiro, T. A., Zeidan, M. A., Lebron-Milad, K., Rodriguez-Romaguera, J., . . . Milad, M. R. (2011). Altered processing of contextual information during fear extinction in PTSD: An fMRI study. *CNS Neuroscience & Therapeutics, 17*(4), 227-236. doi:10.1111/j.1755-5949.2010.00152.x

Saigh, P. A., Yasik, A. E., Oberfield, R. A., Halamandaris, P. V., & Bremner, J. D. (2006). The intellectual performance of traumatized children and adolescents with or without posttraumatic stress disorder. *Journal of Abnormal Psychology, 115*(2), 332-340. doi:10.1037/0021-843x.115.2.232

Samuelson, K. W., Krueger, C. E., Burnett, C., & Wilson, C. K. (2010). Neuropsychological Functioning in Children with Posttraumatic Stress Disorder. *Child Neuropsychology, 16*(2), 119-133. doi:10.1080/09297040903190782

Samuelson, K. W., Neylan, T. C., Lenoci, M., Metzler, T. J., Cardenas, V., Weiner, M. W., & Marmar, C. R. (2009). Longitudinal effects of PTSD on memory functioning. *Journal of International Neuropsychological Society, 15*, 853-861. doi:10.1017/S1355617709990282

Sawamura, T., Klengel, T., Armario, A., Jovanovic, T., Norrholm, S. D., Ressler, K. J., & Andero, R. (2016). Dexamethasone Treatment Leads to Enhanced Fear Extinction and Dynamic Fkbp5 Regulation in Amygdala. *Neuropsychopharmacology, 41*(3), 832-846. doi:10.1038/npp.2015.210

Schafer, I., Langeland, W., Hissbach, J., Luedecke, C., Ohlmeier, M. D., Chodzinski, C., . . . Driessen, M. (2010). Childhood trauma and dissociation in patients with alcohol dependence, drug dependence, or both-A multi-center study. *Drug and Alcohol Dependence, 109*(1-3), 84-89. doi:10.1016/j.drugalcdep.2009.12.012

Schmidt, S. D., Myskiw, J. C., Furini, C. R., Schmidt, B. E., Cavalcante, L. E., & Izquierdo, I. (2015). PACAP modulates the consolidation and extinction of the contextual fear conditioning through NMDA receptors. *Neurobiology of Learning and Memory, 118*, 120-124. doi:10.1016/j.nlm.2014.11.014

Shalev, A. Y., Videlock, E. J., Peleg, T., Segman, R., Pitman, R. K., & Yehuda, R. (2008). Stress hormones and post-traumatic stress disorder in civilian trauma victims: a longitudinal study. Part I: HPA axis responses. *International Journal of Neuropsychopharmacology, 11*(3), 365-372. doi:10.1017/s1461145707008127

Shansky, R. M. (2015). Sex differences in PTSD resilience and susceptibility: Challenges for animal models of fear learning. *Neurobiology of Stress, 1*, 60-65. doi:10.1016/j.ynstr.2014.09.005

Shin, L. M., & Liberzon, I. (2010). The neurocircuitry of fear, stress, and anxiety disorders. *Neuropsychopharmacology, 35*(1), 169-191. doi:10.1038/npp.2009.83

Shvil, E., Sullivan, G. M., Schafer, S., Markowitz, J. C., Campeas, M., Wager, T. D., . . . Neria, Y. (2014). Sex differences in extinction recall in posttraumatic stress disorder: A pilot fMRI study. *Neurobiology of Learning and Memory*. doi:10.1016/j.nlm.2014.02.003

Sijbrandij, M., Engelhard, I. M., Lommen, M. J., Leer, A., & Baas, J. M. (2013). Impaired fear inhibition learning predicts the persistence of symptoms of posttraumatic stress disorder (PTSD). *Journal of Psychiatric Research, 47*(12), 1991-1997. doi:10.1016/j.jpsychires.2013.09.008

Silvestri, A. J., & Root, D. H. (2008). Effects of REM deprivation and an NMDA agonist on the extinction of conditioned fear. *Physiology & Behavior, 93*(1-2), 274-281. doi:10.1016/j.physbeh.2007.08.020

Singewald, N., Schmuckermair, C., Whittle, N., Holmes, A., & Ressler, K. J. (2015). Pharmacology of cognitive enhancers for exposure-based therapy of fear, anxiety and trauma-related disorders. *Pharmacology & Therapeutics, 149*, 150-190. doi:10.1016/j.pharmthera.2014.12.004

Soeter, M., & Kindt, M. (2011). Noradrenergic enhancement of associative fear memory in humans. *Neurobiology of Learning and Memory, 96*(2), 263-271. doi:10.1016/j.nlm.2011.05.003

Soeter, M., & Kindt, M. (2012). Stimulation of the noradrenergic system during memory formation impairs extinction learning but not the disruption of reconsolidation. *Neuropsychopharmacology, 37*(5), 1204-1215. doi:10.1038/npp.2011.307

Soliman, F., Glatt, C. E., Bath, K. G., Levita, L., Jones, R. M., Pattwell, S. S., . . . Casey, B. J. (2010). A genetic variant BDNF polymorphism alters extinction learning in both mouse and human. *Science, 327*(5967), 863-866. doi:10.1126/science.1181886

Soni, M., Curran, V. H., & Kamboj, S. K. (2013). Identification of a narrow post-ovulatory window of vulnerability to distressing involuntary memories in healthy women. *Neurobiology of Learning and Memory, 104*, 32-38. doi:10.1016/j.nlm.2013.04.003

Soravia, L. M., Heinrichs, M., Aerni, A., Maroni, C., Schelling, G., Ehlert, U., . . . de Quervain, D. J. (2006). Glucocorticoids reduce phobic fear in humans. *Proceedings of the National Academy of Science of the United States of America, 103*(14), 5585-5590. doi:10.1073/pnas.0509184103

Soravia, L. M., Heinrichs, M., Winzeler, L., Fisler, M., Schmitt, W., Horn, H., . . . de Quervain, D. J. (2014). Glucocorticoids enhance in vivo exposure-based therapy of spider phobia. *Depression and Anxiety, 31*(5), 429-435. doi:10.1002/da.22219

Southwick, S. M., Morgan Iii, C. A., Charney, D. S., & High, J. R. (1999). Yohimbine use in a natural setting: effects on posttraumatic stress disorder. *Biological Psychiatry, 46*(3), 442-444. doi:10.1016/S0006-3223(99)00107-9

Spoormaker, V. I., Gvozdanovic, G. A., Samann, P. G., & Czisch, M. (2014). Ventromedial prefrontal cortex activity and rapid eye movement sleep are associated with subsequent fear expression in human subjects. *Experimental Brain Research, 232*(5), 1547-1554. doi:10.1007/s00221-014-3831-2

Spoormaker, V. I., Schroter, M. S., Andrade, K. C., Dresler, M., Kiem, S. A., Goya-Maldonado, R., . . . Czisch, M. (2012). Effects of rapid eye movement sleep deprivation on fear extinction recall and prediction error signaling. *Human Brain Mapping, 33*, 2362-2376. doi:10.1002/hbm.21369

Spoormaker, V. I., Sturm, A., Andrade, K. C., Schroter, M. S., Goya-Maldonado, R., Holsboer, F., . . . Czisch, M. (2010). The neural correlates and temporal sequence of the relationship between shock exposure, disturbed sleep and impaired consolidation of fear extinction. *Journal of Psychiatric Research, 44*, 1121-1128. doi:10.1016/j.jpsychires.2010.04.017

Sripada, R. K., Garfinkel, S. N., & Liberzon, I. (2013). Avoidant symptoms in PTSD predict fear circuit activation during multimodal fear extinction. *Frontiers in Human Neuroscience, 7*, 672. doi:10.3389/fnhum.2013.00672

Stickgold, R. (2005). Sleep-dependent memory consolidation. *Nature, 437*(7063), 1272-1278. doi:10.1038/nature04286

Sturm, A., Czisch, M., & Spoormaker, V. I. (2013). Effects of unconditioned stimulus intensity and fear extinction on subsequent sleep architecture in an afternoon nap. *Journal of Sleep Research, 22*(6), 648-655. doi:10.1111/jsr.12074

Szabo, C., Kelemen, O., & Keri, S. (2014). Changes in FKBP5 expression and memory functions during cognitive-behavioral therapy in posttraumatic stress disorder: A preliminary study. *Neuroscience Letters*. doi:10.1016/j.neulet.2014.03.059

Tabbert, K., Merz, C. J., Klucken, T., Schweckendiek, J., Vaitl, D., Wolf, O. T., & Stark, R. (2010). Cortisol enhances neural differentiation during fear acquisition and extinction in contingency aware young women. *Neurobiology of Learning and Memory, 94*(3), 392-401. doi:10.1016/j.nlm.2010.08.006

Taylor, F. B., Lowe, K., Thompson, C., McFall, M. M., Peskind, E. R., Kanter, E. D., . . . Raskind, M. A. (2006). Daytime prazosin reduces psychological distress to trauma specific cues in civilian trauma posttraumatic stress disorder. *Biological Psychiatry, 59*(7), 577-581. doi:10.1016/j.biopsych.2005.09.023

Taylor, F. B., Martin, P., Thompson, C., Williams, J., Mellman, T. A., Gross, C., . . . Raskind, M. A. (2008). Prazosin effects on objective sleep measures and clinical symptoms in civilian trauma posttraumatic stress disorder: A placebo-controlled study. *Biological Psychiatry, 63*(6), 629-632. doi:10.1016/j.biopsych.2007.07.001

Terzioglu, B., Kaleli, M., Aydin, B., Ketenci, S., Cabadak, H., & Goren, M. Z. (2013). Increased noradrenaline levels in the rostral pons can be reversed by M1 antagonist in a rat model of post-traumatic stress disorder. *Neurochemical Research, 38*(8), 1726-1733. doi:10.1007/s11064-013-1076-2

Thakur, G. A., Joober, R., & Brunet, A. (2009). Development and persistence of posttraumatic stress disorder and the 5-HTTLPR polymorphism. *Journal of Traumatic Stress, 22*(3), 240-243. doi:10.1002/jts.20405

Thompson, W. W., & Gottesman, II. (2008). Challenging the conclusion that lower preinduction cognitive ability increases risk for combat-related post-traumatic stress disorder in 2,375 combat-exposed, Vietnam War veterans. *Military Medicine, 173*(6), 576-582.

Toomey, R., Alpern, R., Vasterling, J. J., Baker, D. G., Reda, D. J., Lyons, M. J., . . . Murphy, F. M. (2009). Neuropsychological functioning of U.S. Gulf War veterans 10 years after the war. *Journal of the International Neuropsychological Society, 15*, 717-729. doi:10.1017/S1355617709990294

True, W. R., Rice, J., Eisen, S. A., Heath, A. C., Goldberg, J., Lyons, M. J., & Nowak, J. (1993). A twin study of genetic and environmental contributions to liability for posttraumatic stress symptoms. *Archives of General Psychiatry, 50*(4), 257-264.

Tunbridge, E. M., Harrison, P. J., & Weinberger, D. R. (2006). Catechol-o-methyltransferase, cognition, and psychosis: Val158Met and beyond. *Biological Psychiatry, 60*(2), 141-151. doi:10.1016/j.biopsych.2005.10.024

Uddin, M., Chang, S. C., Zhang, C., Ressler, K., Mercer, K. B., Galea, S., . . . Koenen, K. C. (2013). Adcyap1r1 genotype, posttraumatic stress disorder, and depression among women exposed to childhood maltreatment. *Depression and Anxiety, 30*(3), 251-258. doi:10.1002/da.22037

Vaiva, G., Ducrocq, F., Jezequel, K., Averland, B., Lestavel, P., Brunet, A., & Marmar, C. R. (2003). Immediate treatment with propranolol decreases posttraumatic stress disorder two months after trauma. *Biological Psychiatry, 54*(9), 947-949.

Valente, N. L., Vallada, H., Cordeiro, Q., Bressan, R. A., Andreoli, S. B., Mari, J. J., & Mello, M. F. (2011). Catechol-O-methyltransferase (COMT) val158met polymorphism as a risk factor for PTSD after urban violence. *Journal of Molecular Neuroscience, 43*(3), 516-523. doi:10.1007/s12031-010-9474-2

Van Damme, S., Crombez, G., Hermans, D., Koster, E. H., & Eccleston, C. (2006). The role of extinction and reinstatement in attentional bias to threat: a conditioning approach. *Behaviour Research and Therapy, 44*(11), 1555-1563. doi:10.1016/j.brat.2005.11.008

van Liempt, S., van Zuiden, M., Westenberg, H., Super, A., & Vermetten, E. (2013). Impact of impaired sleep on the development of PTSD symptoms in combat veterans: A prospective longitudinal cohort study. *Depression and Anxiety, 30*(5), 469-474. doi:10.1002/da.22054

van Zuiden, M., Kavelaars, A., Rademaker, A. R., Vermetten, E., Heijnen, C. J., & Geuze, E. (2011). A prospective study on personality and the cortisol awakening response to predict posttraumatic stress symptoms in response to military deployment. *Journal of Psychiatric Research, 45*(6), 713-719. doi:10.1016/j.jpsychires.2010.11.013

VanElzakker, M. B., Kathryn Dahlgren, M., Caroline Davis, F., Dubois, S., & Shin, L. M. (2013). From Pavlov to PTSD: The extinction of conditioned fear in rodents, humans, and anxiety disorders. *Neurobiology of Learning and Memory*. doi:10.1016/j.nlm.2013.11.014

Vasterling, J. J., & Brailey, K. (2005). Neuropsychological findings in adults with PTSD. In J. J. Vasterling & C. R. Brewin (Eds.), *Neuropsychology of PTSD: Biological, Cognitive, and Clinical Perspectives*. New York: The Guilford Press.

Vasterling, J. J., Brailey, K., Constans, J. I., Borges, A., & Sutker, P. B. (1997). Assessment of intellectual resources in Gulf War veterans: Relationship to PTSD. *Assessment, 4*(1), 51-59.

Vasterling, J. J., Brailey, K., Proctor, S. P., Kane, R., Heeren, T., & Franz, M. (2012). Neuropsychological outcomes of mild traumatic brain injury, post-traumatic stress disorder and depression in Iraq-deployed US Army soldiers. *The British Journal of Psychiatry, 201*, 186-192. doi:10.1192/bjp.bp.111.096461

Vasterling, J. J., Duke, L. M., Brailey, K., Constans, J. I., Allain, A. N., & Sutker, P. B. (2002). Attention, learning, and memory performances and intellectual resources in Vietnam veterans: PTSD and no disorder comparisons. *Neuropsychology, 16*(1), 5-14. doi:10.1037//0894-4105.16.1.5

Vasterling, J. J., Proctor, S. P., Amoroso, P., Kane, R., Heeren, T., & White, R. F. (2006). Neuropsychological outcomes of army personnel following deployment to the Iraq war. *Journal of the American Medical Association, 296*(5), 519-529.

Videlock, E. J., Peleg, T., Segman, R., Yehuda, R., Pitman, R. K., & Shalev, A. Y. (2008). Stress hormones and post-traumatic stress disorder in civilian trauma victims: a longitudinal study. Part II: The adrenergic response. *International Journal of Neuropsychopharmacology, 11*(3), 373-380. doi:10.1017/s1461145707008139

Wahbeh, H., & Oken, B. S. (2013). Salivary cortisol lower in posttraumatic stress disorder. *Journal of Traumatic Stress, 26*(2), 241-248. doi:10.1002/jts.21798

Wald, I., Degnan, K. A., Gorodetsky, E., Charney, D. S., Fox, N. A., Fruchter, E., . . . Bar-Haim, Y. (2013). Attention to threats and combat-related posttraumatic stress symptoms: prospective associations and moderation by the serotonin transporter gene. *JAMA Psychiatry, 70*(4), 401-408. doi:10.1001/2013.jamapsychiatry.188

Wegerer, M., Kerschbaum, H., Blechert, J., & Wilhelm, F. H. (2014). Low levels of estradiol are associated with elevated conditioned responding during fear extinction and with intrusive memories in daily life. *Neurobiology of Learning and Memory, 116*, 145-154. doi:10.1016/j.nlm.2014.10.001

Wendt, J., Neubert, J., Lindner, K., Ernst, F. D., Homuth, G., Weike, A. I., & Hamm, A. O. (2014). Genetic influences on the acquisition and inhibition of fear. *International Journal of Psychophysiology*. doi:10.1016/j.ijpsycho.2014.10.007

Wild, J., & Gur, R. C. (2008). Verbal memory and treatment response in post-traumatic stress disorder. *The British Journal of Psychiatry, 193*(3), 254-255. doi:10.1192/bjp.bp.107.045922

Wilker, S., Pfeiffer, A., Kolassa, S., Elbert, T., Lingenfelder, B., Ovuga, E., . . . Kolassa, I. T. (2014). The role of FKBP5 genotype in moderating long-term effectiveness of exposure-based psychotherapy for posttraumatic stress disorder. *Translational Psychiatry, 4*, e403. doi:10.1038/tp.2014.49

Xie, P., Kranzler, H. R., Poling, J., Stein, M. B., Anton, R. F., Farrer, L. A., & Gelernter, J. (2010). Interaction of FKBP5 with childhood adversity on risk for post-traumatic stress disorder. *Neuropsychopharmacology, 35*(8), 1684-1692. doi:10.1038/npp.2010.37

Yehuda, R., Bierer, L. M., Sarapas, C., Makotkine, I., Andrew, R., & Seckl, J. R. (2009). Cortisol metabolic predictors of response to psychotherapy for symptoms of PTSD in survivors of the World Trade Center attacks on September 11, 2001. *Psychoneuroendocrinology, 34*(9), 1304-1313. doi:10.1016/j.psyneuen.2009.03.018

Yehuda, R., Daskalakis, N. P., Desarnaud, F., Makotkine, I., Lehrner, A. L., Koch, E., . . . Bierer, L. M. (2013). Epigenetic Biomarkers as Predictors and Correlates of Symptom Improvement Following Psychotherapy in Combat Veterans with PTSD. *Frontiers in Psychiatry, 4*, 118. doi:10.3389/fpsyt.2013.00118

Yehuda, R., Golier, J. A., Tischler, L., Harvey, P. D., Newmark, R., Yang, R. K., & Buchsbaum, M. S. (2007). Hippocampal volume in aging combat veterans with and without post-traumatic stress disorder: Relation to risk and resilience factors. *Journal of Psychiatric Research, 41*, 435-445. doi:10.1016/j.jpsychires.2005.12.002

Yehuda, R., Morris, A., Labinsky, E., Zemelman, S., & Schmeidler, J. (2007). Ten-year follow-up study of cortisol levels in aging holocaust survivors with and without PTSD. *Journal of Traumatic Stress, 20*(5), 757-761. doi:10.1002/jts.20228

Yehuda, R., Siever, L. J., Teicher, M. H., Levengood, R. A., Gerber, D. K., Schmeidler, J., & Yang, R. K. (1998). Plasma norepinephrine and 3-methoxy-4-hydroxyphenylglycol concentrations and severity of depression in combat posttraumatic stress disorder and major depressive disorder. *Biological Psychiatry, 44*(1), 56-63.

Yehuda, R., Southwick, S., Giller, E. L., Ma, X., & Mason, J. W. (1992). Urinary catecholamine excretion and severity of PTSD symptoms in Vietnam combat veterans. *The Journal of Nervous and Mental Disease, 180*(5), 321-325.

Yehuda, R., Tischler, L., Golier, J. A., Grossman, R., Brand, S. R., Kaufman, S., & Harvey, P. D. (2006). Longitudinal assessment of cognitive performance in Holocaust survivors with and without PTSD. *Biological Psychiatry, 60*(7), 714-721. doi:10.1016/j.biopsych.2006.03.069

Zannas, A. S., & Binder, E. B. (2014). Gene-environment interactions at the FKBP5 locus: sensitive periods, mechanisms and pleiotropism. *Genes, Brain and Behavior, 13*(1), 25-37. doi:10.1111/gbb.12104

Zeidan, M. A., Igoe, S. A., Linnman, C., Vitalo, A., Levine, J. B., Klibanski, A., . . . Milad, M. R. (2011). Estradiol modulates medial prefrontal cortex and amygdala activity during fear extinction in women and female rats. *Biological Psychiatry, 70*(10), 920-927. doi:10.1016/j.biopsych.2011.05.016

Zoladz, P. R., & Diamond, D. M. (2013). Current status on behavioral and biological markers of PTSD: A search for clarity in a conflicting literature. *Neuroscience and Biobehavioral Reviews, 37*(5), 860-895. doi:10.1016/j.neubiorev.2013.03.024

Zuj, D. V., Palmer, M. A., Hsu, C. K., Nicholson, E. L., Cushing, P. J., Gray, K. E., & Felmingham, K. L. (2016). Impaired fear extinction associated with PTSD increases with hours-since-waking. *Depression and Anxiety, 33*(3), 203-210. doi:10.1002/da.22463

Chapter 3

Impaired Fear Extinction Associated with PTSD Increases with Hours-Since-Waking

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3.1 Abstract

Background: Prior research has demonstrated that time-of-day may play an important role in the extinction of conditioned fear, with extinction better learned earlier in the day rather than later. Impaired fear extinction is widely considered a key mechanism of the fear-related features of Posttraumatic stress disorder (PTSD). The relationship between fear extinction and PTSD symptoms may be moderated by hours-since-waking.

Method: In the present experiment, we examined whether hours-since-waking would moderate fear extinction learning ability in a clinical PTSD sample (n = 15), compared to trauma-exposed (n = 33) and non-exposed controls (n = 22). Participants completed a standardized differential fear conditioning and extinction paradigm, providing skin conductance response measures to quantify conditioned responding.

Results: Mixed-model analysis of variance revealed a PTSD-specific impairment in extinction learning ability in the late extinction phase. A moderation analysis showed that hours-since-waking was a significant moderator of the relationship between impaired late extinction and PTSD symptoms. Specifically, we found that participants with higher PTSD symptoms demonstrated poorer fear extinction learning ability as they were awake for longer.

Conclusions: The results of the current study add to a growing literature indicating deficits in fear extinction learning in PTSD samples, compared to trauma-exposed and non-exposed controls. These results support previous findings that fear extinction is impaired later in the day, and extends this to a clinical sample, suggesting that exposure-therapy may be optimized by scheduling sessions in the morning.

3.2 Introduction

Posttraumatic stress disorder (PTSD) is characterized by distressing intrusive memories of a trauma and avoidance behaviors (American Psychiatric Association, 2013). A prominent model posits that impaired fear extinction, the ability to extinguish a conditioned fear association, is an important mechanism underlying PTSD (Pitman et al., 2012). Extinction is the core principle of exposure therapy, the first-line treatment for PTSD (Graham & Milad, 2011), and further investigation is needed to understand the processes that aid or impair extinction. Growing research implicates sleep in extinction learning and consolidation (Pace-Schott, Germain, & Milad, 2015a). Pace-Schott et al. (2013) revealed that sleep prior to extinction learning may enhance extinction, with homeostatic sleep demands increasing throughout the day, and reducing extinction potential. Therefore, hours-since-waking may be an important moderator of extinction learning.

Fear conditioning is central in explaining the development and persistent of PTSD symptoms (Mineka & Oehlberg, 2008). Conditioning and extinction studies reveal PTSD-associated deficits in extinction learning (Blechert, Michael, Vriends, Margraf, & Wilhelm, 2007; Norrholm et al., 2011; Orr et al., 2000; Peri, Ben-Shakhar, Orr, & Shalev, 2000) and extinction recall the following day (Milad et al., 2008; Milad et al., 2009; Shvil et al., 2014). Longitudinal studies assessing pre-trauma extinction reveal impaired extinction learning predicts increased PTSD symptoms after trauma (Guthrie & Bryant, 2006; Orr et al., 2012), supporting the role of extinction learning in PTSD development.

Growing evidence suggests an important role of sleep in extinction consolidation (Germain, 2013; Pace-Schott, Germain, et al., 2015a; Pace-Schott, Germain, & Milad, 2015b). The first study to examine such effects found that after extinction learning, a night of sleep led to the generalization of an extinguished CS+ to a previously unextinguished CS+, compared to a period of wakefulness (Pace-Schott et al., 2009). Additionally, participants

who achieved rapid eye movement (REM) sleep during a nap following extinction showed significantly smaller skin conductance response (SCR) to the extinguished CS+ after sleep, compared to participants who did not achieve REM sleep during the nap (Spoormaker et al., 2010). Evidence in patients with spider phobia found a reduction in spider fear following exposure therapy was significantly greater after a period of sleep than wakefulness (Kleim et al., 2014), confirming an earlier study of simulated exposure therapy for spider fear where sleep enhanced extinction retention and generalization compared to wakefulness (Pace-Schott, Verga, Bennett, & Spencer, 2012).

Sleep disturbances may contribute to issues in managing stressors, enhancing vulnerability for developing psychiatric disorders, including PTSD (Bryant, Creamer, O'Donnell, Silove, & McFarlane, 2010). Indeed, prospective studies have found pre-, peri-, and post-trauma sleep disturbances significantly predict PTSD symptoms (Bryant et al., 2010; Kobayashi, Sledjeski, Spoonster, Fallon, & Delahanty, 2008; Koren, Arnon, Lavie, & Klein, 2002; Mellman, Pigeon, Nowell, & Nolan, 2007; van Liempt, van Zuiden, Westenberg, Super, & Vermetten, 2013; Wright et al., 2011). The sleep-wake cycle is regulated by the suprachiasmatic nucleus of the hypothalamus (Schwartz & Roth, 2008) and structures in the brainstem, hypothalamic and basal forebrain arousal networks (Saper, Fuller, Pedersen, Lu, & Scammell, 2010), and hypothalamic and brainstem networks are also dysregulated in PTSD (Felmingham et al., 2010). Further, amygdala and prefrontal activation impact sleep, and these networks are also dysregulated in PTSD (Germain, Buysse, & Nofzinger, 2008), suggesting a neurobiological relationship between these factors.

Time-of-day has a significant impact on extinction learning, with improved extinction and recall in the morning compared to the evening in healthy men (Pace-Schott, Germain, et al., 2015a; Pace-Schott et al., 2013). Generalization of an extinguished stimulus to an unextinguished stimulus was also better 24-hours later if both learning and recall occurred in

the morning when an individual is likely to be well rested. Pace-Schott et al. (2014) hypothesized that increased homeostatic sleep pressure may explain greater psychophysiological reactivity to conditioned stimuli in the evening, emphasizing the influence of the previous nights' sleep for extinction. During sleep deprivation, adenosine levels increase in the basal forebrain to promote sleep, and it is hypothesized that increasing adenosine levels may influence sleep homeostasis; that is, the increasing need for restorative sleep throughout the day (Porkka-Heiskanen & Kalinchuk, 2011). Pace-Schott, Germain, et al. (2015a) suggest strategically timed sleep may promote the consolidation of therapeutic extinction learning to maximize treatment benefit. These findings are limited to healthy men and yet to be examined in a clinically anxious population characterized by impaired fear extinction and impaired sleep, such as PTSD.

On the basis of accumulating evidence of PTSD-specific extinction impairments, we predicted participants with PTSD would have significantly impaired fear extinction learning compared to trauma-exposed and trauma-non-exposed controls. Furthermore, based on the recent finding of Pace-Schott et al. (2013) we hypothesized that hours-since-waking would be a significant moderator between fear extinction learning and PTSD symptoms, with poorer fear extinction learning predicting greater PTSD symptom severity, and that this relationship would become stronger with increased hours-since-waking.

3.3 Method

3.3.1 Participants.

Seventy participants aged 18-63 (M = 24.2 years, SD = 9.3 years; 25 males and 45 females) were tested between 12-6PM, and comprised three groups: PTSD (n = 15), trauma-exposed (TC; n = 33), and non-trauma-exposed controls (NTC; n = 22). Participants were classified on the basis of exposure to a criterion A stressor which threatened physical

integrity (American Psychiatric Association, 2000) using the Traumatic Events Questionnaire (TEQ; Vrana and Lauterbach (1994); war exposure n = 1; accident n = 13; natural disaster n = 18; witness n = 29; assaulted or molested n = 21; threatened or held captive n = 15; and tortured or terrorist victim n = 1). Mean years since trauma was 7.6 years (SD = 10.7 years). Individuals who reported never having experienced a traumatic event were classified as NTC. The PTSD Checklist-Civilian version (PCL-C; Weathers, Litz, Huska, & Keane, 1994) was used to estimate PTSD caseness, with PTSD participants classified as those presenting with at least 1 intrusive symptom, 3 avoidance symptoms, and 2 hyperarousal symptoms (roughly equating to a total PCL-C score ≥ 40; Weathers et al., 1994; National Center for Posttraumatic Stress Disorder, n.d.) Participants who displayed minimal PTSD symptoms were allocated to the TC group. The study was conducted in accordance with the Declaration of Helsinki, the design was approved by the University of Tasmania Social Sciences Human Research Ethics Committee, and all participants gave full informed consent.

3.3.2 Questionnaires.

The PCL-C (Weathers et al., 1994) was used for probable PTSD diagnosis according to DSM-IV criteria, and to provide a continuous measure of symptom severity. Participants also completed the Depression Anxiety Stress Scale 21-item version (DASS; Lovibond & Lovibond, 1995), Pittsburgh Sleep Quality Index (PSQI; Buysse, Reynolds, Monk, Berman, & Kupfer, 1989) and the Alcohol Use Disorder Identification Test (AUDIT; Babor, Higgins-Biddle, Saunders, & Monteiro, 2001). Upon arrival to the lab, participants indicated their time-of-waking to derive the number of hours-since-waking.

3.3.3 Stimuli and experimental protocol.

We adopted a differential fear conditioning and extinction paradigm used previously (Orr et al., 2000). The unconditioned stimulus (US) was a 500ms mild electric shock delivered by an electrode to the first interosseous muscle of the dominant hand, set at a level considered "highly annoying, but not painful" (Orr et al., 2000). Conditioned stimuli were red and blue circles presented individually for 12s on a computer screen. The protocol included four experimental phases: *habituation, acquisition, early extinction*, and *late extinction*. During *habituation*, participants were exposed to four trials of each coloured circle (8 trials in total). During *acquisition*, one of the coloured circles was followed by the US (CS+) on all 5 trials (100% reinforcement schedule; Orr et al., 2000) while the other coloured circle was not reinforced on any of the five trials (CS-; 10 trials in total). The *early extinction* phase consisted of 5 trials of the CS+ (with no reinforcement) and 5 trials of the CS- (10 trials in total) followed by the *late extinction* phase, which mirrored early extinction. Trial order was fixed-random, with no more than two consecutive CS+ or CS- trials.

3.3.4 Skin conductance response.

Skin conductance level (SCL) was measured through a $22mV_{rms}$, 75Hz constant-voltage coupler (FE116, ADInstruments, Sydney, Australia) with bipolar electrodes placed on the intermediate phalange of the first and third finger of the non-dominant hand, sampled at 512Hz and stored at 64Hz, and recorded in micro-Siemens (μS). Skin conductance response (SCR) to the CS+ and CS- was calculated by subtracting the mean SCL during the 2s prior to CS onset from the maximum SCR during the 12s CS duration. Scoring was conducted by a software macro blind to experimental conditions. SCR values were square-root transformed, and the absolute value of negative values was transformed and the negative sign replaced. Differential conditioned responding (DCR) was calculated by subtracting the

SCR of the first CS- from the first CS+, and so on for all trials (as in Menz et al., 2013). DCR scores were used to compute an extinction DCR change score for use in the moderation analysis, by subtracting the trial 5 DCR from the trial 1 DCR, with greater values indicating a greater decline in differential fear responding.

3.3.5 US-expectancy ratings.

During the 12s stimulus presentation, participants were asked to rate their threat expectancy of the US on a 0-100 visual analogue scale (VAS; 0 "certain no electrical stimulus"; 100 "certain electrical stimulus"; as previously used by Lommen et al., 2013).

3.3.6 Statistical analyses.

To assess fear conditioning and extinction, a 3(group) × 2(CS) × 5(trial) mixed analysis of variance (ANOVA) was conducted on SCR data separately for each experimental phase (four trials for habituation and acquisition). The first CS+/- trials were removed from acquisition analyses as the US had not been encountered, and no learning had occurred. Successful fear conditioning was assessed with a significant CS main effect with greater SCR to the CS+ compared to the CS- during acquisition. Successful extinction learning was assessed with a significant CS × trial interaction, with SCRs decreasing over trials for early and late extinction, and with reduced discrimination between the CS+ and CS-. Mixed model ANOVAs were identical for SCR data and US-expectancy ratings[1], with the exception of the first CS+/- trial for SCR data being omitted from analyses. A moderation analysis was conducted using the PROCESS macro for SPSS (model 1; Hayes, 2013) with hours-since-

[1] Five participants were omitted from analyses involving US-expectancy ratings due to missing data. The sample size for US-expectancy analyses is $N = 65$ (PTSD: $n = 12$; TC: $n = 31$; NTC: $n = 22$). Due to the low sample size of the PTSD group in particular, the results of these analyses should be interpreted with caution.

waking as the moderator variable, extinction DCR change score as the predictor variable, and PCL total as the outcome variable.

An alpha level of $\alpha = .05$ was used for all tests of significance (two-tailed). Greenhouse-Geisser adjustments were made for within-subjects variables, with epsilon values and adjusted-df reported where necessary. Brown-Forsythe *F*-ratio corrections were made when homogeneity of variance was violated. For pairwise comparisons, Bonferroni corrections were made, with effect sizes reported as Cohen's *d*, following criteria of 0.2, 0.5, and 0.8 for small, moderate, and large effects, respectively (Cohen, 1988). For mixed model ANOVAs, effect sizes were reported as partial eta-squared (η_p^2).

3.4 Results

3.4.1 Clinical and demographic data.

Table 1 displays descriptive and inferential statistics for clinical and demographic variables. A one-way ANOVA revealed a significant between-group effect of age, $F(2, 18.43) = 7.28$, $p = .005$. As seen in Table 1, participants in the PTSD group were, on average, significantly older than participants in the TC and NTC groups (ps < .05), who did not differ ($p = .375$). ANOVA revealed a significant between-group effect for total PCL score, $F(2, 18.30) = 76.44$, $p < .001$, with the PTSD group having a greater PCL total ($M = 50.60$, 95% CI[44.15, 57.05], $SD = 11.65$) than TCs ($M = 25.00$ [23.40, 26.60], $SD = 4.52$), and NTCs ($M = 19.91$ [18.73, 21.09], $SD = 2.65$), who also showed a significant difference ($p < .001$). Table 1 shows the PTSD group had significantly greater PCL scores for each symptom cluster. One-way ANOVA revealed participants in the PTSD group had significantly higher scores on the DASS subscales of depression, anxiety and stress than TC and NTC participants, who did not significantly differ (ps > .05). Participants in the PTSD group had significantly poorer sleep quality compared to TC and NTC participants, who did not

significantly differ (p = .187). There were no significant between-group differences in alcohol use (p = .124).

Table 1

Mean Scores and SDs of Demographic and Clinical Variables.

Measures	PTSD (n = 15)	TC (n = 33)	NTC (n = 22)	Test Statistic	p
Demographic Data					
- Age (years)	32.87 (14.95)	22.52 (6.13)	20.82 (3.13)	$F_{(2, 18.43)} = 7.28$	$p = .005$
- Sex	8F, 7M	23F, 10M	14F, 8M	$\chi^2_{(2)} = 1.21$	$p = .546$
PCL-C					
- Intrusive	3.07 (1.16)	0.24 (0.61)	0.00 (0.00)	$F_{(2, 67)} = 109.54$	$p < .001$
- Avoidance	3.93 (2.15)	0.70 (0.85)	0.18 (0.50)	$F_{(2, 18.44)} = 34.12$	$p < .001$
- Hyperarousal	3.40 (0.99)	0.48 (0.87)	0.18 (0.50)	$F_{(2, 37.15)} = 82.25$	$p < .001$
DASS					
- Depression	7.80 (5.27)	1.91 (2.10)	1.14 (1.57)	$F_{(2, 19.50)} = 17.84$	$p < .001$
- Anxiety	7.63 (4.54)	2.18 (1.92)	1.23 (1.76)	$F_{(2, 21.57)} = 20.39$	$p < .001$
- Stress	12.23 (5.49)	4.33 (2.53)	2.43 (2.13)	$F_{(2, 22.32)} = 30.89$	$p < .001$
PSQI Total	8.60 (4.52)	5.31 (2.16)	4.31 (1.91)	$F_{(2, 23.49)} = 8.25$	$p = .002$
AUDIT	7.87 (5.82)	5.13 (3.68)	5.23 (4.40)	$F_{(2, 65)} = 2.15$	$p = .124$

Note: PCL-C = PTSD Checklist-Civilian version; DASS = Depression Anxiety Stress Scale; PSQI = Pittsburgh Sleep Quality Index; AUDIT = Alcohol Use Disorders Identification Test.

3.4.2 Hours-since-waking.

Between-group differences in hours-since-waking showed no indication of group biases for hours-since-waking as a moderator: A one-way ANOVA revealed no significant between-group differences in hours-since-waking, $F(2, 67) = 1.24$, $p = .296$ (PTSD group = 7:02hrs, *SD* = 2:30; TC = 6:09, *SD* = 2:26; and NTC = 5:43, *SD* = 2:32). Further, a one-way ANOVA revealed no significant group differences for time-of-day when testing was conducted, $F(2, 67) = .34$, $p = .711$ (PTSD group = 14:43PM, *SD* = 1:42; TC = 15:04PM, *SD* = 2:09; and NTC = 14:40PM, *SD* = 1:45), indicating that circadian rhythm is unlikely to affect analyses involving hours-since-waking.

3.4.3 Fear conditioning and extinction (SCR)

Habituation. A 3 × 2 × 4 mixed-ANOVA showed no significant main effect of group or CS condition or interactions with these variables (*F*s < 1.07). There was a significant main effect of trial, $F(3, 201) = 5.52$, $p = .001$, $\eta_p^2 = .076$, with SCRs decreasing across trials (see Figure 1A). There is likely a reduction in SCRs as participants become used to the task, reflecting an initial habituation of responses that is commonly observed.

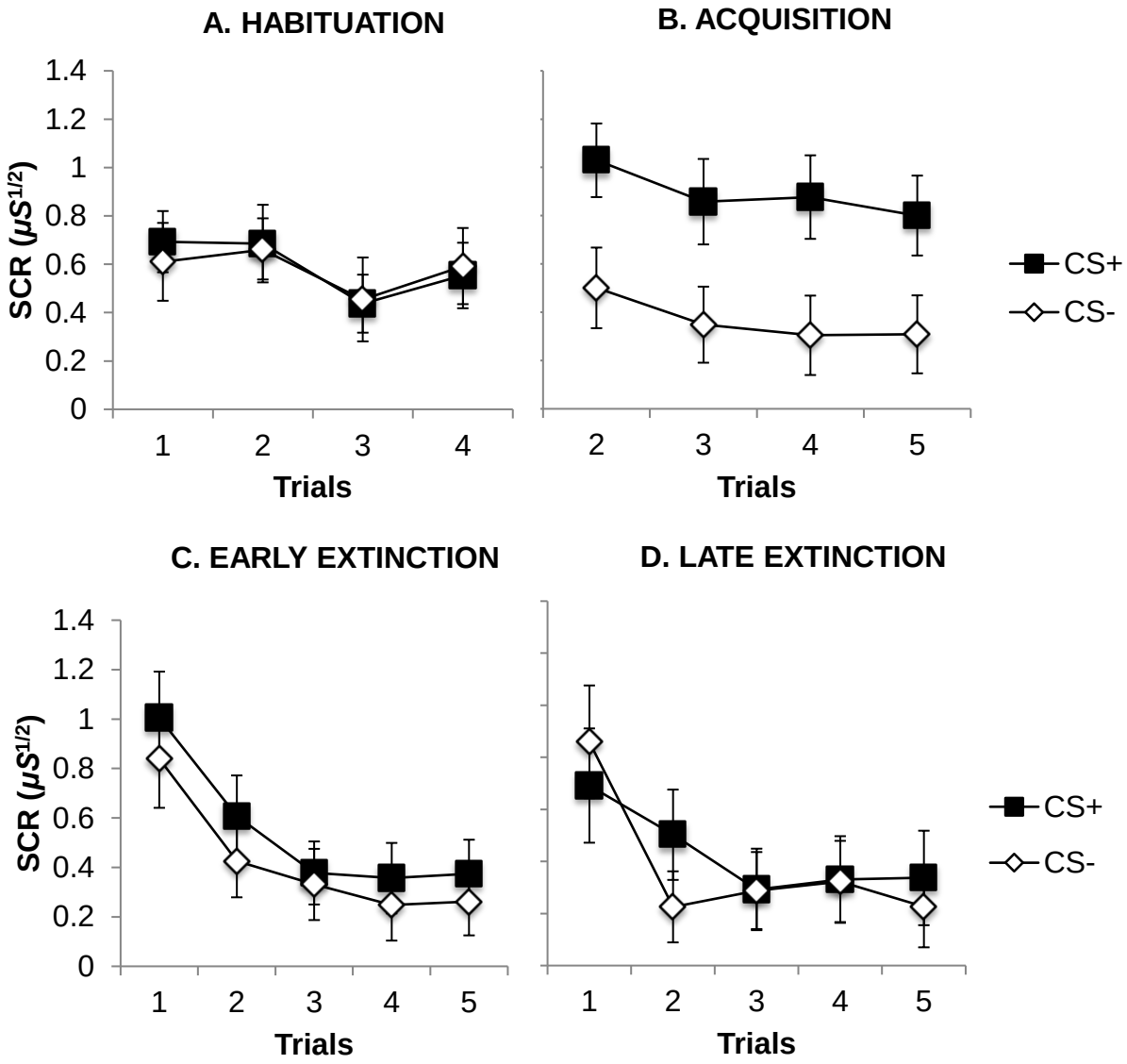

Figure 1. CS × Trial interactions for each experimental phase. Fear acquisition and extinction scores pooled across group for the CS+ and CS- across all trials. **(A)** During the acquisition phase, there was a statistically significant CS × trial interaction, with the difference in responses to the CS+ and CS- increasing across the acquisition phase, indicating greater CS+/- contingency awareness. The first CS+/- trials were removed from the acquisition phase, as the US had not been encountered and no learning had occurred. **(C)** In early extinction, there was no significant CS × trial interaction, with means showing that the reduction of SCR from trial 1 to trial 5 did not differ between the CS+ and CS-. **(D)** During

the late extinction phase, there was a trend for a CS × trial interaction, $F(3.74, 250.75) = 2.42$, $p = .053$, $\eta_p^2 = .035$, $\varepsilon = .936$, with a greater reduction in SCR from trial 1 to trial 2 for the CS- compared to the CS+. Error bars depict 95% confidence intervals of the mean. $\mu S^{1/2}$, SCR square-root transformed in micro-siemens.

Acquisition. The successful acquisition of a conditioned response to the CS+ is evidenced by a significant main effect of CS, $F(1, 67) = 69.29$, $p < .001$, $d = 0.84$. Mixed-model ANOVA revealed the CS+ elicited a significantly larger SCR ($M = 0.89$ [0.76, 1.02], $SD = 0.55$) than the CS- ($M = 0.37$ [0.25, 0.48], $SD = 0.49$). There was a significant main effect of trial, $F(2.68, 179.41) = 3.81$, $p = .014$, $\eta_p^2 = .054$, $\varepsilon = .893$. Additionally, ANOVA revealed a significant group main effect, $F(2, 67) = 4.09$, $p = .021$, $\eta_p^2 = .109$, with the TC group showing larger SCR ($M = 0.81$ [0.66, 0.96], $SD = 0.43$) compared to PTSD and NTC groups ($M = 0.59$ [0.37, 0.81], $SD = 0.43$; and $M = 0.49$ [0.31, 0.67], $SD = 0.43$, respectively). However, there were no significant group × CS, or group × trial interactions, indicating that responses to the CS+ and CS- did not differ between groups.

Early extinction. Mixed ANOVA revealed a significant main effect of CS, with the CS+ eliciting a larger SCR ($M = 0.54$ [0.44, 0.65], $SD = 0.44$) than the CS- ($M = 0.42$ [0.33, 0.51], $SD = 0.39$), $F(1, 67) = 7.57$, $p = .008$, $d = 0.29$. There was no significant group × CS interaction ($p = .243$), indicating all groups were still displaying conditioned responding during the early extinction phase. ANOVA also revealed a significant main effect of trial, with a decline in SCR across the experimental phase, $F(3.29, 220.44) = 27.99$, $p < .001$, $\eta_p^2 = .295$, $\varepsilon = .823$, indicating an overall reduction in SCRs across trials (see Figure 1C). Further, there was no significant CS × trial interaction, $F(3.78, 253.37) = 0.37$, $p = .819$, $\eta_p^2 = .006$, ε

= .945, with no change in SCR to the CS+ and CS-, providing additional support that all groups were still displaying conditioned responses to the CS+ in early extinction (see Figure 1C). No further main effects or interactions were significant (Fs < 1).

Late extinction. There was a significant trial main effect, with SCRs decreasing across trials, $F(3.11, 208.53) = 16.82$, $p < .001$, $\eta_p^2 = .201$, $\varepsilon = .778$. There was no significant main effect of CS, $F(1, 67) = 1.16$, $p = .295$, $d = 0.08$, and a trend for a CS × trial interaction, $F(3.74, 250.75) = 2.43$, $p = .053$, $\eta_p^2 = .035$, $\varepsilon = .936$, suggesting a reduction in conditioned responding for all groups by the end of late extinction. There was a trend for a CS × trial interaction, $F(3.74, 250.75) = 2.42$, $p = .053$, $\eta_p^2 = .035$, $\varepsilon = .936$, with a greater reduction in SCR from trial 1 to trial 2 for the CS- compared to the CS+. Importantly, the mixed ANOVA revealed a significant group × CS × trial interaction, $F(7.49, 250.75) = 2.43$, $p = .018$, $\eta_p^2 = .068$, $\varepsilon = .936$. Tests of simple interaction effects revealed a significant CS × trial interaction for the PTSD group only, $F(2.44, 34.16) = 4.26$, $p = .017$, $\eta_p^2 = .233$, $\varepsilon = .610$. As seen in Figure 2A, the PTSD group displayed a temporary increase in SCR to the CS+, followed by an increase in responding to the CS-. Notably, this interaction was only identified in the PTSD group, with no effects found for TCs and NTCs ($p = .184$ and $p = .585$, respectively), indicating a PTSD-specific impairment in extinguishing conditioned responses.

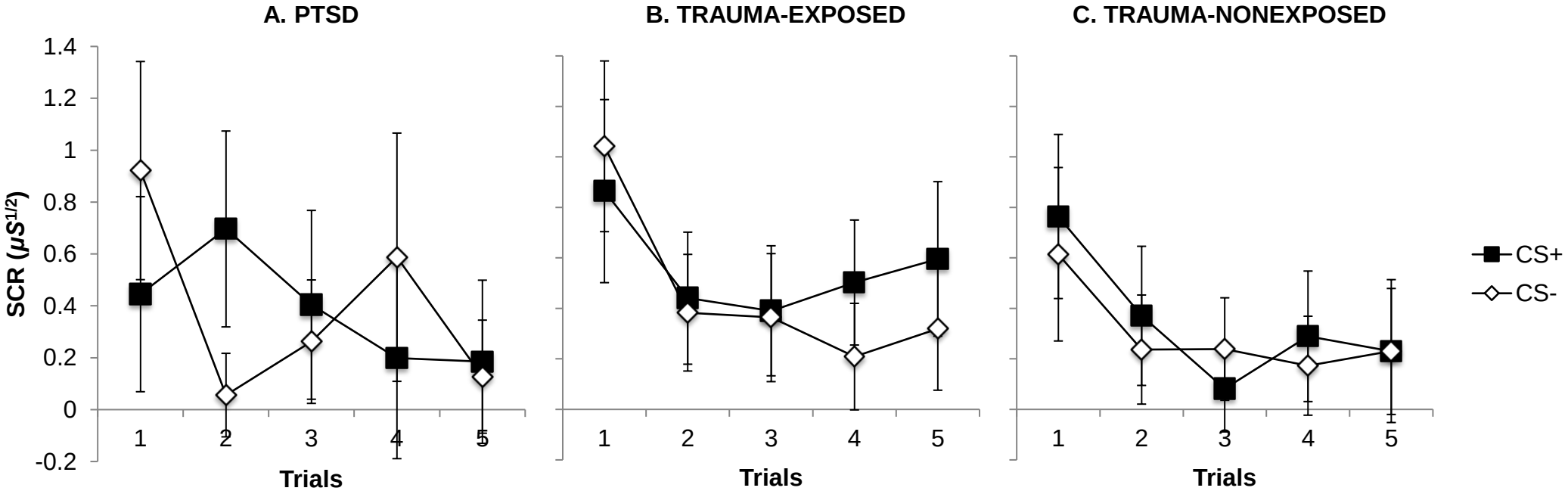

Figure 2. Group × CS × Trial interaction in late extinction. Late extinction SCR scores for the CS+ and CS- by trial for each: **(A)** PTSD; **(B)** TC; and **(C)** NTC groups. The PTSD group showed reduced extinction learning, with the CS+ eliciting a temporary increase in SCR, followed by an SCR increase to the CS-. CS × trial interactions for the TC and NTC groups were not significant (p = .184 and p = .585, respectively). Error bars depict 95% confidence intervals of the mean. $\mu S^{1/2}$, SCR square-root transformed in micro-siemens.

3.4.4 Threat expectancy.

Habituation. No significant main effects or interactions were identified during the habituation phase.

Acquisition. Mixed ANOVA revealed a significant CS main effect, $F(1, 62) = 459.27$, $p < .001$, $d = 4.43$, and trial main effect, $F(3.25, 201.24) = 5.19$, $p = .001$, $\eta_p^2 = .077$, $\varepsilon = .811$. Further, there was a significant CS × trial interaction, $F(3.07, 190.20) = 103.01$, $p < .001$, $\eta_p^2 = .624$, $\varepsilon = .767$, with increased differential responding between the CS+ and CS- over the course of the acquisition phase. No further main effects or interactions were significant.

Early extinction. ANOVA revealed a significant CS main effect, $F(1, 62) = 42.51$, $p < .001$, $d = 0.87$, and a significant main effect of trial, $F(2.58, 160.01) = 66.44$, $p < .001$, $\eta_p^2 = .517$, $\varepsilon = .645$. There was also a significant CS × trial interaction, $F(3.30, 204.41) = 6.60$, $p < .001$, $\eta_p^2 = .096$, $\varepsilon = .824$, US-expectancies to the CS+ and CS- decreasing over the early extinction phase.

Late extinction. Mixed-model ANOVA revealed a significant main effect of CS, $F(1, 62) = 9.48$, $p = .003$, $d = 0.60$, with greater US-expectancy to the CS+ ($M = 28.30$ [22.31, 34.29], $SD = 24.19$) compared to the CS- ($M = 19.94$ [14.47, 25.41], $SD = 22.09$). Further, there was a significant trial main effect, $F(1.87, 116.15) = 47.29$, $p < .001$, $\eta_p^2 = .433$, $\varepsilon = .468$, with US-expectancies reducing from trial 1 ($M = 43.89$ [37.66, 50.11], $SD = 25.10$) to trial 5 ($M = 14.72$ [9.34, 20.10], $SD = 21.69$).

3.4.5 Hours-since-waking moderation.

As a significant group effect was only found during late extinction, the DCR change score from this phase was calculated and entered into the moderation model as the predictor variable, with PCL total as the outcome variable, and hours-awake as the moderator (Table 2, Model 1). The model predicted a significant amount of variance in PCL scores, $R^2 = .18$, $F(3, 66) = 4.93$, $p = .004$. Table 2 shows late extinction DCR change was a significant predictor of PCL scores, with a trend for the role of hours-awake, and a significant hours-awake × late extinction DCR change interaction. Age, depression, anxiety and stress were individually included in the model as covariates with little change to inferential statistics, and no change to the pattern of effects (see Supporting Information; Appendix C).

Table 2

Linear Models of Predictors of PCL Total.

	b	*SE b*	*t*	*p*
Model 1				
- Constant	28.88 [25.97, 31.80]	1.46	19.79	< .001
- Hours-awake	1.14 [-0.05, 2.32]	0.59	1.91	.060
- Late extinction DCR change	-2.37 [-4.68, -0.06]	1.16	-2.05	.045
- Hours-awake × late extinction	-1.18 [-2.07, -0.29]	0.45	-2.63	.011
Model 2				
- Constant	28.62 [25.67, 31.57]	1.48	19.38	< .001
- Hours-awake	1.22 [-0.11, 2.54]	0.66	1.84	.071
- CS+ SCR change	-2.47 [-5.63, 0.69]	1.58	-1.56	.124
- Hours-awake × CS+ change	-1.80 [-3.29, -0.30]	0.75	-2.40	.019

Note: SE = standard error. Square brackets show 95% confidence intervals of *b*.

As shown in Figure 3, for participants with higher PTSD symptom severity, increased hours-awake was significantly associated with poorer extinction learning (as indexed by a smaller decline in DCR change over the late extinction phase), and this effect becomes stronger with increasing hours-awake. To ensure that increased responding to the CS- in trial 1 for the PTSD group did not drive this effect, a change score was calculated for the CS+ in late extinction (trial 5 SCR subtracted from trial 1 SCR) and included as the predictor variable. Table 2 (Model 2) shows a significant moderation interaction, with *b*-values revealing a consistent pattern with Model 1.

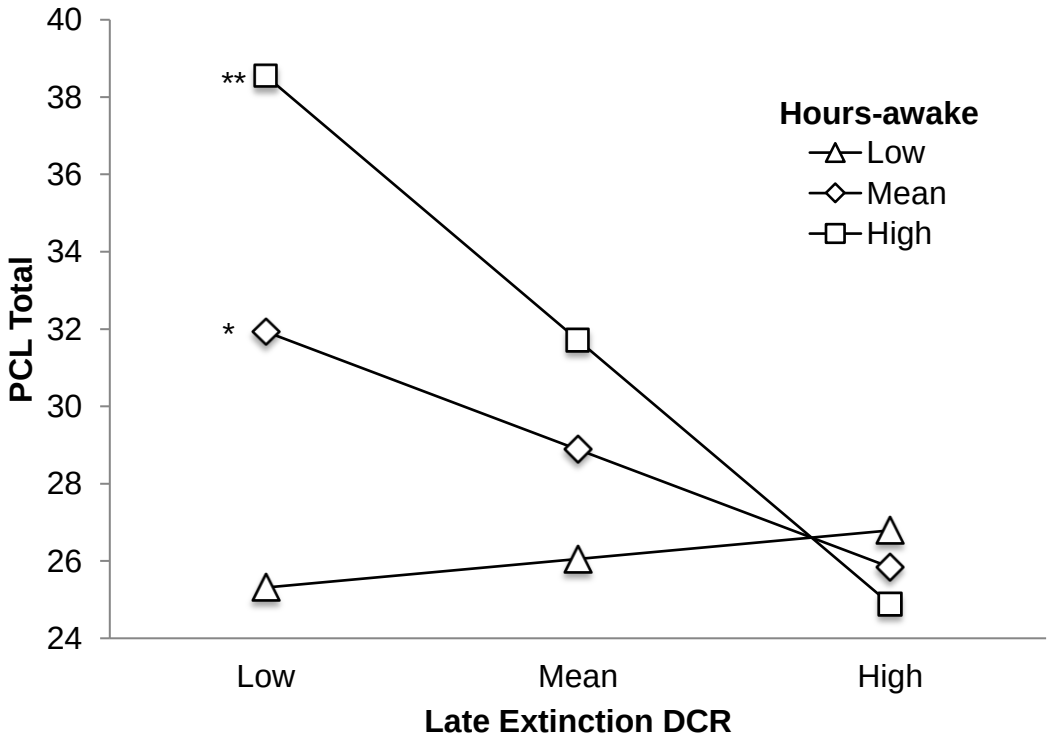

Figure 3. Hours-awake moderation. The relationship between hours-awake, late extinction DCR change and PCL total was significant at higher levels (+1 *SD*) of hours-awake (8.70 hours, *b* = -5.31 [-8.75, -1.87], *t* = -3.08, *p* = .003), and mean levels (6.21 hours, *b* = -2.37 [-4.68, -0.06], *t* = -2.05, *p* = .045), with no significant effects at lower levels (-1 *SD*) of hours-awake (3.71 hours, *b* = 0.57 [-2.39, 3.54], *t* = 0.39, *p* = .700). These findings indicate a linear

relationship between late extinction DCR change and PCL total, at increasing levels of hours-since-waking, whereby participants with higher PCL scores demonstrate lower change in differential fear responding in the late extinction phase, and this relationship becomes stronger as participants are awake for longer. * $p < .05$, ** $p < .01$.

3.5 Discussion

This study found hours-since-waking moderates the relationship between fear extinction and PTSD symptoms. Participants with higher PCL scores show significantly poorer extinction learning with increasing hours-awake. These findings support research identifying poorer extinction learning in the evening in healthy controls (Pace-Schott et al., 2013), and extends them to a clinical sample with PTSD compared to TC and NTC. Together, these results have important clinical implications for scheduling exposure therapy to optimize treatment benefit.

The findings of the present study are consistent with previous research that fear extinction learning is significantly impaired in PTSD compared with nonclinical controls (Blechert et al., 2007; Norrholm et al., 2011; Orr et al., 2000; Peri et al., 2000). The results highlight an interesting pattern, with extinction learning impairments limited to the late extinction phase, with no between-group differences during early extinction. This pattern of findings is novel and may reflect early consolidation processes. Further investigations with varying extinction learning and recall periods (e.g., 2-day paradigms; Milad et al., 2008; Milad et al., 2009; Shvil et al., 2014) may provide some insight into the consolidation of extinction recall. Further, it should be noted that the PCL estimates probable PTSD diagnosis, and future research would benefit from using a Clinician Administered PTSD Scale.

Alternatively, participants in the PTSD group may develop increased state anxiety in anticipation of the US, despite clear learning of the CS+/- contingency during acquisition. During late extinction, participants in the PTSD group temporarily show larger SCR to the CS- compared to the CS+, suggesting fear generalization from the CS+ to the CS-. However, this effect dissipates quickly, which may be a result of the 100% reinforcement schedule during acquisition. The use of a partial reinforcement schedule (e.g., 60%; Milad et al., 2007; Pace-Schott et al., 2013) may shed light on the mechanisms of fear generalization.

Pace-Schott et al. (2013) found extinction learning was more effective in the morning compared to the evening in healthy males. The current results show higher PCL scores are negatively associated with extinction learning as participants are awake for longer, extending previous findings to a clinical sample of males and females. This supports the notion that extinction is better learned soon after sleep compared to later in the day, with greater physiological reactivity to stimuli partially attributed to increased sleep demands (Pace-Schott et al., 2014). This idea is supported by research showing enhanced extinction learning and greater generalization of recall memories soon after sleep (Pace-Schott et al., 2009; Pace-Schott et al., 2013; Pace-Schott et al., 2014; Pace-Schott et al., 2012), particularly REM sleep (Spoormaker, Gvozdanovic, Samann, & Czisch, 2014; Spoormaker et al., 2010).

In the current study, we found hours-since-waking moderates the relationship between fear extinction and PTSD symptoms. Previous research revealed elevated circadian levels of cortisol and testosterone in the morning, which may explain significantly enhanced extinction learning and recall in the morning (Pace-Schott et al., 2013). PTSD is often characterized by hypocortisolism (Yehuda & Seckl, 2011), and increased cortisol levels enhance extinction learning (Bentz et al., 2013; Merz, Hamacher-Dang, & Wolf, 2014). Cortisol experiences circadian effects (van Zuiden et al., 2011), suggesting the cortisol acrophase in the morning may be one mechanism by which hours-awake impacts extinction in PTSD. Further, the

PTSD group was, on average, significantly older than the TC and NTC groups. There was a weak negative correlation between age and late extinction DCR change, and including age as a covariate in the moderation analysis did not alter the findings (see Supporting Information; Appendix C). This implies that age does not significantly influence the effects in the current study, however should be controlled in future research.

While the absence of physiological measures of sleep and waking are a limitation of the current study, our findings highlight the role that sleep plays in fear extinction ability. Self-report measures of sleep using the PSQI revealed the PTSD group reported significantly poorer sleep quality compared to control groups. Poorer sleep quality in the PTSD group may reduce the restorative benefits of sleep, thereby increasing homeostatic sleep demands and producing a smaller window early in the day for maximum extinction potential. Further, recent evidence found extinction is learned faster and better generalized in the morning in participants who achieve sleep earlier and report higher-morningness (Pace-Schott, Rubin, et al., 2015), suggesting chronotype may influence the present study's findings. Currently, the relationship between REM sleep and PTSD diagnosis is somewhat inconclusive (Germain, 2013), however further polysomnographic sleep studies examining REM sleep as a moderator between fear extinction and PTSD symptoms may clarify this relationship. Further, although there were no significant between-group differences in testing time, circadian rhythm was not directly examined in the current study and should be included in future investigations into the effects of hours-awake.

3.5.1 Clinical implications.

Pace-Schott et al. (2012) suggested the effectiveness of exposure therapy could be improved by scheduling treatment soon after sleep. The implications of the present study are in accordance with previous recommendations that a period of sleep shortly before or after

exposure therapy may aid in therapeutic extinction learning (Kleim et al., 2014; Pace-Schott et al., 2012). This notion is supported by a wealth of research proposing that sleep aids in memory consolidation (e.g., Diekelmann, 2014; Diekelmann & Born, 2010).

3.6 Conclusion

The present study confirms impaired fear extinction learning associated with PTSD, and extends previous findings to reveal that this relationship is moderated by hours-since-waking, with PTSD-related extinction impairments increasing with time-awake. These findings suggest important implications for scheduling exposure-based treatments for PTSD, indicating that extinction learning potential, and thus benefit from exposure therapy, may decrease throughout the day. Future research would benefit from physiological measures of sleep to determine a role that REM sleep plays in enhancing this window of opportunity for optimized treatment benefit.

3.7 References

American Psychiatric Association. (2000). *Diagnostic and Statistical Manual of Mental Disorders* (4th ed.). Washington, D.C: American Psychiatric Association.

American Psychiatric Association. (2013). *Diagnostic and Statistical Manual of Mental Disorders* (5th ed.). Washington, DC: Author.

Babor, T. F., Higgins-Biddle, J. C., Saunders, J. B., & Monteiro, M. G. (2001). *The Alcohol Use Disorders Identification Test: Guidelines for use in primary care* (2nd ed.): World Health Organisation: Department of Mental Health and Substance Dependence.

Bentz, D., Michael, T., Wilhelm, F. H., Hartmann, F. R., Kunz, S., von Rohr, I. R. R., & de Quervain, D. J. F. (2013). Influence of stress on fear memory processes in an aversive differential conditioning paradigm in humans. *Psychoneuroendocrinology, 38*(7), 1186-1197. doi:10.1016/j.psyneuen.2012.12.018

Blechert, J., Michael, T., Vriends, N., Margraf, J., & Wilhelm, F. H. (2007). Fear conditioning in posttraumatic stress disorder: Evidence for delayed extinction of autonomic, experiential, and behavioural responses. *Behaviour Research and Therapy, 45*(9), 2019-2033. doi:10.1016/j.brat.2007.02.012

Bryant, R. A., Creamer, M., O'Donnell, M., Silove, D., & McFarlane, A. C. (2010). Sleep disturbance immediately prior to trauma predicts subsequent psychiatric disorder. *Sleep, 33*(1), 69-74.

Buysse, D. J., Reynolds, C. F., Monk, T. H., Berman, S. R., & Kupfer, D. J. (1989). The Pittsburgh Sleep Quality Index (PSQI): A new instrument for psychiatric research and practice. *Psychiatry Research, 28*(2), 193-213.

Cohen, J. (1988). *Statistical power analysis for the behavioural sciences* (2nd ed.). Hillsdale, NJ: Lawrence Erlbaum.

Diekelmann, S. (2014). Sleep for cognitive enhancement. *Frontiers in Systems Neuroscience, 8*, 46. doi:10.3389/fnsys.2014.00046

Diekelmann, S., & Born, J. (2010). The memory function of sleep. *Nature Reviews Neuroscience, 11*(2), 114-126. doi:10.1038/nrn2762

Felmingham, K., Williams, L. M., Kemp, A. H., Liddell, B., Falconer, E., Peduto, A., & Bryant, R. (2010). Neural Responses to Masked Fear Faces: Sex Differences and Trauma Exposure in Posttraumatic Stress Disorder. *Journal of Abnormal Psychology, 119*(1), 241-247.

Germain, A. (2013). Sleep disturbances as the hallmark of PTSD: where are we now? *The American Journal of Psychiatry, 170*(4), 372-382. doi:10.1176/appi.ajp.2012.12040432

Germain, A., Buysse, D. J., & Nofzinger, E. (2008). Sleep-specific mechanisms underlying posttraumatic stress disorder: Integrative review and neurobiological hypotheses. *Sleep Medicine Reviews, 12*, 185-195. doi:10.1016/j.smrv.2007.09.003

Graham, B. M., & Milad, M. R. (2011). The study of fear extinction: Implications for anxiety disorders. *The American Journal of Psychiatry, 168*(12), 1255-1265. doi:10.1176/appi.ajp.2011.11040557

Guthrie, R. M., & Bryant, R. A. (2006). Extinction learning before trauma and subsequent posttraumatic stress. *Psychosomatic Medicine, 68*, 307-311. doi:10.1097/01.psy.0000208629.67653.cc

Hayes, A. F. (2013). *Introduction to mediation, moderation, and conditional process analysis: A regression-based approach*. New York: The Guilford Press.

Kleim, B., Wilhelm, F. H., Temp, L., Margraf, J., Wiederhold, B. K., & Rasch, B. (2014). Sleep enhances exposure therapy. *Psychological Medicine*, 1-9. doi:10.1017/s0033291713001748

Kobayashi, I., Sledjeski, E. M., Spoonster, E., Fallon, W. F., Jr., & Delahanty, D. L. (2008). Effects of early nightmares on the development of sleep disturbances in motor vehicle accident victims. *Journal of Traumatic Stress, 21*(6), 548-555. doi:10.1002/jts.20368

Koren, D., Arnon, I., Lavie, P., & Klein, E. (2002). Sleep complaints as early predictors of posttraumatic stress disorder: A 1-year prospective study of injured survivors of motor vehicle accidents. *The American Journal of Psychiatry, 159*, 855-857.

Lovibond, S. H., & Lovibond, P. F. (1995). *Manual for the Depression Anxiety Stress Scales* (2nd ed.). Sydney: Psychology Foundation.

Mellman, T. A., Pigeon, W. R., Nowell, P. D., & Nolan, B. (2007). Relationships between REM sleep findings and PTSD symptoms during the early aftermath of trauma. *Journal of Traumatic Stress, 20*(5), 893-901. doi:10.1002/jts.20246

Menz, M. M., Rihm, J. S., Salari, N., Born, J., Kalisch, R., Pape, H. C., . . . Buchel, C. (2013). The role of sleep and sleep deprivation in consolidating fear memories. *Neuroimage, 75*, 87-96. doi:10.1016/j.neuroimage.2013.03.001

Merz, C. J., Hamacher-Dang, T. C., & Wolf, O. T. (2014). Exposure to stress attenuates fear retrieval in healthy men. *Psychoneuroendocrinology, 41*, 89-96. doi:10.1016/j.psyneuen.2013.12.009

Milad, M. R., Orr, S. P., Lasko, N. B., Chang, Y., Rauch, S. L., & Pitman, R. K. (2008). Presence and acquired origin of reduced recall for fear extinction in PTSD: Results of a twin study. *Journal of Psychiatric Research, 42*(7), 515-520. doi:10.1016/j.jpsychires.2008.01.017

Milad, M. R., Pitman, R. K., Ellis, C. B., Gold, A. L., Shin, L. M., Lasko, N. B., . . . Rauch, S. L. (2009). Neurobiological basis of failure to recall extinction memory in posttraumatic stress disorder. *Biological Psychiatry, 66*(12), 1075-1082. doi:10.1016/j.biopsych.2009.06.026

Milad, M. R., Wright, C. I., Orr, S. P., Pitman, R. K., Quirk, G. J., & Rauch, S. L. (2007). Recall of fear extinction in humans activates the ventromedial prefrontal cortex and hippocampus in concert. *Biological Psychiatry, 62*(5), 446-454. doi:10.1016/j.biopsych.2006.10.011

Mineka, S., & Oehlberg, K. (2008). The relevance of recent developments in classical conditioning to understanding the etiology and maintenance of anxiety disorders. *Acta Psychologica, 127*(3), 567-580. doi:10.1016/j.actpsy.2007.11.007

National Center for Posttraumatic Stress Disorder. (n.d.). Using the PTSD Checklist for DSM-IV (PCL). Retrieved from http://www.ptsd.va.gov/professional/pages/assessments/assessment-pdf/PCL-handout.pdf

Norrholm, S. D., Jovanovic, T., Olin, I. W., Sands, L. A., Karapanou, I., Bradley, B., & Ressler, K. J. (2011). Fear extinction in traumatized civilians with posttraumatic stress disorder: Relation to symptom severity. *Biological Psychiatry, 69*(6), 556-563. doi:10.1016/j.biopsych.2010.09.013

Orr, S. P., Lasko, N. B., Macklin, M. L., Pineles, S. L., Chang, Y., & Pitman, R. K. (2012). Predicting post-trauma stress symptoms from pre-trauma psychophysiologic reactivity, personality traits and measures of psychopathology. *Biology of Mood & Anxiety Disorders, 2*(8).

Orr, S. P., Metzger, L. J., Lasko, N. B., Macklin, M. L., Peri, T., & Pitman, R. K. (2000). De novo conditioning in trauma-exposed individuals with and without posttraumatic stress disorder. *Journal of Abnormal Psychology, 109*(2), 290-298. doi:10.1037/0021-843x.109.2.290

Pace-Schott, E. F., Germain, A., & Milad, M. R. (2015a). Effects of sleep on memory for conditioned fear and fear extinction. *Psychological Bulletin*. doi:10.1037/bul0000014

Pace-Schott, E. F., Germain, A., & Milad, M. R. (2015b). Sleep and REM sleep disturbance in the pathophysiology of PTSD: the role of extinction memory. *Biology of Mood & Anxiety Disorders, 5*, 3. doi:10.1186/s13587-015-0018-9

Pace-Schott, E. F., Milad, M. R., Orr, S. P., Rauch, S. L., Stickgold, R., & Pitman, R. K. (2009). Sleep promotes generalization of extinction of conditioned fear. *Sleep, 32*(1), 19-26.

Pace-Schott, E. F., Rubin, Z. S., Tracy, L. E., Spencer, R. M., Orr, S. P., & Verga, P. W. (2015). Emotional trait and memory associates of sleep timing and quality. *Psychiatry Research, 229*(3), 999-1010. doi:10.1016/j.psychres.2015.05.069

Pace-Schott, E. F., Spencer, R. M. C., Vijayakumar, S., Ahmed, N. A. K., Verga, P. W., Orr, S. P., . . . Milad, M. R. (2013). Extinction of conditioned fear is better learned and recalled in the morning than in the evening. *Journal of Psychiatric Research, 47*(11), 1776-1784. doi:10.1016/j.jpsychires.2013.07.027

Pace-Schott, E. F., Tracy, L. E., Rubin, Z., Mollica, A. G., Ellenbogen, J. M., Bianchi, M. T., . . . Orr, S. P. (2014). Interactions of time of day and sleep with between-session habituation and extinction memory in young adult males. *Experimental Brain Research, 232*(5), 1443-1458. doi:10.1007/s00221-014-3829-9

Pace-Schott, E. F., Verga, P. W., Bennett, T. S., & Spencer, R. M. C. (2012). Sleep promotes consolidation and generalization of extinction learning in simulated exposure therapy for spider fear. *Journal of Psychiatric Research, 46*, 1036-1044. doi:10.1016/j.jpsychires.2012.04.015

Peri, T., Ben-Shakhar, G., Orr, S. P., & Shalev, A. Y. (2000). Psychophysiologic assessment of aversive conditioning in posttraumatic stress disorder. *Biological Psychiatry, 47*(6), 512-519. doi:10.1016/s0006-3223(99)00144-4

Pitman, R. K., Rasmusson, A. M., Koenen, K. C., Shin, L. M., Orr, S. P., Gilbertson, M. W., . . . Liberzon, I. (2012). Biological studies of post-traumatic stress disorder. *Nature, 13*, 769-787. doi:10.1038/nm3339

Porkka-Heiskanen, T., & Kalinchuk, A. V. (2011). Adenosine, energy metabolism and sleep homeostasis. *Sleep Medicine Reviews, 15*(2), 123-135. doi:10.1016/j.smrv.2010.06.005

Saper, C. B., Fuller, P. M., Pedersen, N. P., Lu, J., & Scammell, T. E. (2010). Sleep state switching. *Neuron, 68*(6), 1023-1042. doi:10.1016/j.neuron.2010.11.032

Schwartz, J. R., & Roth, T. (2008). Neurophysiology of sleep and wakefulness: basic science and clinical implications. *Current Neuropharmacology, 6*(4), 367-378. doi:10.2174/157015908787386050

Shvil, E., Sullivan, G. M., Schafer, S., Markowitz, J. C., Campeas, M., Wager, T. D., . . . Neria, Y. (2014). Sex differences in extinction recall in posttraumatic stress disorder: A pilot fMRI study. *Neurobiology of Learning and Memory*. doi:10.1016/j.nlm.2014.02.003

Spoormaker, V. I., Gvozdanovic, G. A., Samann, P. G., & Czisch, M. (2014). Ventromedial prefrontal cortex activity and rapid eye movement sleep are associated with subsequent fear expression in human subjects. *Experimental Brain Research, 232*(5), 1547-1554. doi:10.1007/s00221-014-3831-2

Spoormaker, V. I., Sturm, A., Andrade, K. C., Schroter, M. S., Goya-Maldonado, R., Holsboer, F., . . . Czisch, M. (2010). The neural correlates and temporal sequence of the relationship between shock exposure, disturbed sleep and impaired consolidation of fear extinction. *Journal of Psychiatric Research, 44*, 1121-1128. doi:10.1016/j.jpsychires.2010.04.017

van Liempt, S., van Zuiden, M., Westenberg, H., Super, A., & Vermetten, E. (2013). Impact of impaired sleep on the development of PTSD symptoms in combat veterans: A prospective longitudinal cohort study. *Depression and Anxiety, 30*(5), 469-474. doi:10.1002/da.22054

van Zuiden, M., Kavelaars, A., Rademaker, A. R., Vermetten, E., Heijnen, C. J., & Geuze, E. (2011). A prospective study on personality and the cortisol awakening response to predict posttraumatic stress symptoms in response to military deployment. *Journal of Psychiatric Research, 45*(6), 713-719. doi:10.1016/j.jpsychires.2010.11.013

Vrana, S. R., & Lauterbach, D. (1994). Prevalence of traumatic events and post-traumatic psychological symptoms in a nonclinical sample of college students. *Journal of Traumatic Stress, 7*, 289-302.

Weathers, F. W., Litz, B. T., Huska, J. A., & Keane, T. M. (1994). *The PTSD Checklist-Civilian Version (PCL-C) for DSM-IV*. Boston: National Center for PTSD, Behavioral Sciences Division.

Wright, K. M., Britt, T. W., Bliese, P. D., Adler, A. B., Picchioni, D., & Moore, D. (2011). Insomnia as predictor versus outcome of PTSD and depression among Iraq combat veterans. *Journal of Clinical Psychology, 67*(12), 1240-1258. doi:10.1002/jclp.20845

Yehuda, R., & Seckl, J. (2011). Minireview: Stress-related psychiatric disorders with low cortisol levels: a metabolic hypothesis. *Endocrinology, 152*(12), 4496-4503. doi:10.1210/en.2011-1218

3.8 Online Supporting Information

S1 – Habituation

S2 – Acquisition

S3 – Early extinction

S4 – Late extinction

S5 – Age Correlations with DCR Change Scores

Differential Conditioned Response (DCR) scores were created by subtracting the CS- trial 1 SCR from the CS+ trial 1 SCR, and so on for every trial. Group × trial analyses were conducted for each phase.

S1. Habituation.

A 3 (group) × 4 (trial) mixed-model ANOVA revealed no significant group or trial main effects, and no significant group × trial interaction (Fs < 1, ps > .569).

S2. Acquisition.

The DCR score from trial 1 was omitted from analyses of acquisition as the unconditioned stimulus (US) had not yet been encountered, and thus no learning had occurred on these trials. A 3 (group) × 4 (trial) mixed-model ANOVA revealed no significant group or trial main effects, and no significant group × trial interaction (Fs < 1.14, ps > .340).

S3. Early extinction.

A 3 (group) × 5 (trial) mixed-model ANOVA revealed no significant group or trial main effects, and no significant group × trial interaction (Fs < 1.45, ps > .243).

S4. Late extinction.

A 3 (group) × 5 (trial) mixed-model ANOVA revealed no significant main effect of group, $F(2, 67) = 0.44$, $p = .647$, $\eta_p^2 = .013$. ANOVA revealed a trend for a trial main effect, $F(3.74, 250.75) = 2.42$, $p = .053$, $\eta_p^2 = .035$, $\varepsilon = .936$, and a significant group × trial interaction, $F(7.49, 250.75) = 2.43$, $p = .018$, $\eta_p^2 = .068$, $\varepsilon = .936$. Test of simple main effects revealed a significant main effect of trial for the PTSD group, $F(2.44, 34.16) = 4.26$, $p = .017$, $\eta_p^2 = .233$, $\varepsilon = .610$, with no significant main effects for the TC and NTC groups ($p = .184$ and $p = .585$, respectively). Inspection of the means revealed that the PTSD group experienced an increase in DCR from trial 1 to trial 2 ($M = -.48$, to $M = .641$, respectively), followed by a decrease to trial 4 ($M = -.388$), and a further increase to trial 5 ($M = .06$).

S5. Age correlations with DCR change scores

Pearson's correlations were conducted between age and the DCR change scores for the acquisition, early extinction and late extinction phases. During acquisition and early extinction there were no significant correlations, Pearson's $r(N = 70) = -.02$, $p = .856$, and $r(N = 70) = .02$, $p = .856$, respectively. There was a trend for a weak negative correlation between age and late extinction DCR scores, $r(N = 70) = -.21$, $p = .078$, indicating a pattern whereby increasing age is associated with a reduction in differential extinction learning during this phase. However this is a weak correlation, and including age as a covariate in moderation analyses did not change the results, or alter the pattern of findings.

Table S1

Linear Model of Predictors of PCL Total (Age Covariate).

	b	*SE b*	*t*	*p*
Constant	13.10	5.23	2.50	.015
	[2.65, 23.56]			
Hours-awake	0.66	0.59	1.11	.270
	[-0.52, 1.84]			
Late Extinction DCR	3.79	2.73	1.39	.170
	[-1.66, 9.24]			
Hours-awake × Late Extinction	-0.86	0.44	-1.95	.055
	[-1.73, 0.02]			
Age (covariate)	0.47	0.16	2.91	.005
	[0.15, 0.79]			

Note: With age included as a covariate, the model predicted a significant amount of variance in PCL scores, $R^2 = .28$, $F(4, 65) = 6.23$, $p < .001$. Further inspection of the interaction revealed that as hours-awake increased, the relationship between PCL scores and extinction learning became stronger. Specifically, the relationship between hours-awake, late extinction DCR and PCL total was only significant at higher levels (+1 SD) of hours-awake (b = -3.66 [-7.12, -0.21], t = -2.12, p = .038), compared to mean levels (b = -1.53 [-3.79, 0.74], t = -1.35, p = .183) and lower levels (-1 SD; b = 0.61 [-2.20, 3.42], t = 0.43, p = .666).

SE = standard error. Square brackets show 95% confidence intervals of *b*.

Table S2

Linear Model of Predictors of PCL Total (Depression Covariate).

	b	*SE b*	*t*	*p*
Constant	18.50 [15.59, 24.41]	2.96	6.25	< .001
Hours-awake	0.52 [-0.35, 1.40]	0.44	1.19	.237
Late Extinction DCR	2.42 [-1.64, 6.47]	2.03	1.19	.238
Hours-awake × Late Extinction	-0.68 [-1.32, -0.03]	-2.10	-2.10	.039
Depression (covariate)	2.26 [1.72, 2.81]	8.25	8.25	< .001

Note: With depression included as a covariate, the model predicted a significant amount of variance in PCL scores, R^2 = .60, $F(4, 65) = 24.45$, $p < .001$. Further inspection of the interaction revealed that as hours-awake increased, the relationship between PCL scores and extinction learning became stronger. Specifically, the relationship between hours-awake, late extinction DCR and PCL total was significant at higher levels (+1 SD) of hours-awake (*b* = -3.46 [-5.93, -1.00], *t* = -2.81, *p* = .007), and mean levels (*b* = -1.78 [-3.41, -0.15], *t* = -2.18, *p* = .033), but was not significant at lower levels (-1 SD; *b* = -0.09 [-2.19, 2.00], *t* = -0.09, *p* = .930).

SE = standard error. Square brackets show 95% confidence intervals of *b*.

Table S3

Linear Model of Predictors of PCL Total (Anxiety Covariate).

	b	*SE b*	*t*	*p*
Constant	16.25 [11.11, 21.39]	2.57	6.31	< .001
Hours-awake	0.62 [-0.14, 1.37]	0.38	1.64	.106
Late Extinction DCR	2.02 [-1.46, 5.50]	1.74	1.16	.251
Hours-awake × Late Extinction	-0.55 [-1.10, 0.001]	0.28	-1.99	.051
Anxiety (covariate)	2.76 [2.25, 3.27]	0.75	10.75	< .001

Note: With anxiety included as a covariate, the model predicted a significant amount of variance in PCL scores, R^2 = .71, $F(4, 65) = 39.01$, $p < .001$. Further inspection of the interaction revealed that as hours-awake increased, the relationship between PCL scores and extinction learning became stronger. Specifically, the relationship between hours-awake, late extinction DCR and PCL total was significant at higher levels (+1 SD) of hours-awake (b = -2.78 [-4.91, -0.65], t = -2.60, p = .011), compared to mean levels (b = -1.41 [-2.81, 0.002], t = -1.99, p = .050), and lower levels (-1 SD; b = -0.03 [-1.82, 1.77], t = -0.03, p = .975).

SE = standard error. Square brackets show 95% confidence intervals of *b*.

Table S4

Linear Model of Predictors of PCL Total (Stress Covariate).

	b	*SE b*	*t*	*p*
Constant	12.43 [7.65, 17.21]	2.40	5.19	< .001
Hours-awake	0.70 [0.02, 1.37]	0.38	2.07	.043
Late Extinction DCR	0.79 [-2.36, 3.95]	1.58	0.50	.618
Hours-awake × Late Extinction	-0.21 [-0.72, 0.29]	0.25	-0.84	.404
Stress (covariate)	2.21 [1.86, 2.55]	0.17	12.61	< .001

Note: With stress included as a covariate, the model predicted a significant amount of variance in PCL scores, $R^2 = .76$, $F(4, 65) = 43.92$, $p < .001$. Further inspection of the interaction revealed that as hours-awake increased, the relationship between PCL scores and extinction learning became stronger. However, differences were not significant at higher levels (+1 SD) of hours-awake (b = -1.07 [-3.05, 0.92], t = -1.08, p = .286), mean levels (b = -0.54 [-1.82, 0.75], t = -0.83, p = .409), or lower levels (-1 SD; b = -0.002 [-1.61, 1.61], t = -0.002, p = .998).

SE = standard error. Square brackets show 95% confidence intervals of *b*.

Table S5

Linear Model of Predictors of PCL Total (Baseline Cortisol Covariate).

	b	*SE b*	*t*	*p*
Constant	29.59	1.70	17.45	< .001
	[26.20, 32.98]			
Hours-awake	1.15	0.61	1.89	.064
	[-0.07, 2.37]			
Late Extinction DCR	1.17	1.17	-2.06	.044
	[-4.75, -0.07]			
Hours-awake × Late Extinction	-1.19	0.45	-2.63	.011
	[-2.09, -0.29]			
Baseline Cortisol (covariate)	-3.06	4.45	-0.69	.494
	[-11.95, 5.83]			

Note: With baseline cortisol included as a covariate, the model predicted a significant amount of variance in PCL scores, R^2 = .19, $F(4, 64) = 3.67$, p = .009. Further inspection of the interaction revealed that as hours-awake increased, the relationship between PCL scores and extinction learning became stronger. Specifically, the relationship between hours-awake, late extinction DCR and PCL total was significant at higher levels (+1 SD) of hours-awake (b = -5.39 [-8.88, -1.89], t = -3.08, p = .003), and mean levels (b = -2.41 [-4.75, -0.07], t = -2.06, p = .044), but was not significant at lower levels (-1 SD; b = 0.56 [-2.42, 3.55], t = 0.38, p = .708).

SE = standard error. Square brackets show 95% confidence intervals of *b*.

Table S6

Linear Model of Predictors of PCL Total (Post-Acquisition Cortisol Covariate).

	b	*SE b*	*t*	*p*
Constant	28.72 [24.77, 32.68]	1.98	14.50	< .001
Hours-awake	1.07 [-0.14, 2.28]	0.61	1.76	.083
Late Extinction DCR	-2.47 [-4.82, -0.12]	1.18	-2.10	.040
Hours-awake × Late Extinction	-1.17 [-2.07, -0.26]	0.45	-2.58	.012
Post-acquisition cortisol (covariate)	1.70 [-13.33, 16.73]	7.52	0.23	.822

Note: With post-acquisition cortisol included as a covariate, the model predicted a significant amount of variance in PCL scores, R^2 = .18, $F(4, 64) = 3.54$, p = .011. Further inspection of the interaction revealed that as hours-awake increased, the relationship between PCL scores and extinction learning became stronger. Specifically, the relationship between hours-awake, late extinction DCR and PCL total was significant at higher levels (+1 SD) of hours-awake (b = -5.39 [-8.90, -1.88], t = -3.07, p = .003), and mean levels (b = -2.47 [-4.82, -0.12], t = -2.10, p = .040), but was not significant at lower levels (-1 SD; b = 0.45 [-2.55, 3.45], t = 0.30, p = .765).

SE = standard error. Square brackets show 95% confidence intervals of *b*.

Table S7

Linear Model of Predictors of PCL Total (Cortisol Reactivity Covariate).

	b	*SE b*	*t*	*p*
Constant	29.18	1.46	19.97	< .001
	[26.26, 32.09]			
Hours-awake	1.28	0.61	2.12	.038
	[0.08, 2.49]			
Late Extinction DCR	-2.46	1.15	-2.14	.036
	[-4.76, -0.17]			
Hours-awake × Late Extinction	-1.23	0.45	-2.76	.008
	[-2.12, -0.34]			
Cortisol Reactivity (Covariate)	13.80	8.50	1.62	.109
	[-3.18, 30.78]			

Note: With cortisol reactivity included as a covariate, the model predicted a significant amount of variance in PCL scores, R^2 = .21, $F(4, 64) = 4.33$, p = .004. Further inspection of the interaction revealed that as hours-awake increased, the relationship between PCL scores and extinction learning became stronger. Specifically, the relationship between hours-awake, late extinction DCR and PCL total was significant at higher levels (+1 SD) of hours-awake (b = -5.53 [-8.98, -2.09], t = -3.21, p = .002), and mean levels (b = -2.46 [-4.76, -0.17], t = -2.14, p = .036), but was not significant at lower levels (-1 SD; b = 0.60 [-2.33, 3.54], t = 0.41, p = .682).

SE = standard error. Square brackets show 95% confidence intervals of *b*.

Chapter 4

Negative Appraisals and Fear Extinction are Independently Related to PTSD Symptoms

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4.1 Abstract

Background: Considerable research has revealed impaired fear extinction to be a significant predictor of PTSD. Fear extinction is also considered the primary mechanism of exposure therapy, and a critical factor in PTSD recovery. The cognitive theory of PTSD proposes that symptoms persist due to excessive negative appraisals about the trauma and its sequelae. Research has not yet examined the relationship between fear extinction and negative appraisals in PTSD.

Methods: A cross-sectional sample of PTSD (n = 21), trauma-exposed (n = 33), and non-exposed participants (n = 26) underwent a standardized differential fear conditioning and extinction paradigm, with skin conductance response (SCR) serving as the index of conditioned responses. The Posttraumatic Cognitions Inventory (PTCI) was used to index catastrophic negative appraisals.

Results: Participants with PTSD demonstrated significantly slower extinction learning and greater negative appraisals compared to control groups. A moderation analysis revealed that both negative trauma-relevant appraisals and fear extinction learning were independently associated with PTSD symptoms, but there was no moderation interaction.

Limitations: The current study was limited by a modest sample size, leading to the inclusion of participants with subclinical PTSD symptoms. Further, the current study only assessed fear extinction learning, and including a second day extinction recall task may show alternative effects.

Conclusions: These findings indicate that negative appraisals and fear extinction did not interact in PTSD, but had independent effects. Here we show for the first time in an experimental framework that negative appraisals and fear extinction play separate roles in PTSD symptoms.

4.2 Introduction

Posttraumatic stress disorder (PTSD) is characterized by persistent fear, intrusive memories, and negative appraisals about oneself and a traumatic event (Dunmore, Clark, & Ehlers, 1999). Symptoms naturally subside in many trauma survivors, but persist as PTSD in approximately 2-10% of people (Atwoli, Stein, Koenen, & McLaughlin, 2015; Ramchand et al., 2010). The cognitive theory of PTSD (Ehlers & Clark, 2000) proposes that two key mechanisms underlie the persistence of PTSD: negative appraisals relating to the trauma and its sequelae, and poorly elaborated autobiographical memories and conditioned fear responses which readily prime intrusive memories that are triggered by trauma reminders (Ehlers & Clark, 2000).

A considerable body of research supports the idea that PTSD develops and is maintained by negative cognitions about a significant traumatic event (Dunmore et al., 1999; Dunmore, Clark, & Ehlers, 2001; Ehlers, Mayou, & Bryant, 1998, 2003). That is, traumatic experiences lead to negative appraisals about the trauma and its sequelae, causing feelings of current threat, persistent avoidance, and generalization of fear (Ehlers & Clark, 2000). Numerous studies have shown acute post-trauma negative appraisals significantly predict increased PTSD symptoms in adults and children following a range of traumatic events (e.g., Dunmore et al., 2001; Ehlers et al., 1998, 2003; Ehring, Ehlers, & Glucksman, 2008). Moreover, the tendency to engage in negative appraisals prior to a traumatic event predicts PTSD symptoms several years after exposure (Bryant & Guthrie, 2007).

A second influential model proposes that PTSD develops from impaired fear extinction learning and recall (Pitman et al., 2012). In this model, fear conditioning occurs during trauma, and re-exposure to trauma reminders and subsequent avoidance of reminders facilitate conditioned fear and fear generalization. Extinction typically occurs when the conditioned stimuli are presented in the absence of any aversive consequence, and in the

context of trauma this typically involves experience of benign trauma reminders. In this sense, the minority of trauma survivors who experience persistent symptoms can be regarded as suffering impaired extinction (Davis & Myers, 2002). Considerable evidence demonstrates that individuals presenting with PTSD show impairments in fear extinction and extinction recall (e.g., Milad et al., 2008; Norrholm et al., 2011; Shvil et al., 2014; Zuj et al., 2016). Recent studies have also found pre-trauma extinction learning significantly predicts increased post-traumatic stress (Guthrie & Bryant, 2006; Lommen, Engelhard, Sijbrandij, van den Hout, & Hermans, 2013; Orr et al., 2012).

These models are not mutually exclusive, given that Ehlers and Clark (2000) also suggest that intrusive memories and conditioned fear responses triggered by trauma reminders are thought to reinforce negative appraisals, which act to maintain anxiety and sense of current threat. Further, longitudinal evidence has found that PTSD symptoms can be predicted by pre-trauma fear extinction learning (Guthrie & Bryant, 2006), and maladaptive pre-trauma negative appraisals (Bryant & Guthrie, 2007). Therefore, impaired extinction capacity pre-trauma and heightened negative appraisals may interact to potentiate fear responses following trauma. In an experimental framework, negative appraisals may moderate the relationship between fear extinction and PTSD symptoms. To our knowledge, no previous studies have examined fear extinction and negative appraisals in tandem, in relation to PTSD.

On the basis of Ehlers and Clark's (2000) model, we predicted first that excessively negative appraisals (indexed by the Posttraumatic Cognitions Inventory (PTCI)) would be associated with increased PTSD symptom severity. Second, on the basis of considerable cross-sectional and longitudinal evidence we hypothesized that impaired fear extinction learning would be associated with PTSD symptoms. As yet, studies have not considered these two prevailing models in tandem, thus moderation analyses were conducted testing for a

possible interaction between fear extinction learning and negative appraisals in influencing PTSD symptom severity.

4.3 Method

4.3.1 Participants.

Eighty participants aged 18-63 years (*M* = 27.8 years, *SD* = 12.8 years; 33 males and 47 females) comprised three groups: PTSD (*n* = 21), trauma-exposed without PTSD (TC; *n* = 33), and non-trauma exposed controls (NTC; *n* = 26). The PTSD and TC groups were classified on the basis of experiencing a criterion A stressor, whereby physical integrity was threatened (American Psychiatric Association, 2000) using the Traumatic Events Questionnaire (TEQ; Vrana & Lauterbach, 1994). Traumatic events included war-related combat (*n* = 5), life-threatening accident (*n* = 21), natural disaster (*n* = 26), witnessing a traumatic event (*n* = 34), assaulted or molested (*n* = 21), threatened or held captive (*n* = 13), and tortured or terrorist victim (*n* = 2). Mean years since trauma for the PTSD group was 10.1 years (*SD* = 12.8 years) and 10.5 years (*SD* = 11 years) for the TC group. Trauma-exposed individuals were classified into either the PTSD or TC group using the PTSD Checklist (PCL-C) for DSM-IV (Weathers, Litz, Huska, & Keane, 1994). The PCL-C for DSM-IV was used as recruitment occurred prior to diagnostic instruments for the DSM-V being available. The University of Tasmania Social Sciences ethics committee approved the study protocol. Informed consent of the participants was obtained after the nature of the procedures had been fully explained.

4.3.2 Measures.

PTSD Checklist-Civilian version. The PCL-C (Weathers et al., 1994) is a 17-item self-report measure that provides diagnostic information according to PTSD criteria on the DSM-IV (American Psychiatric Association, 2000). Responses are made on a 5-point Likert scale in regards to how often participants were distressed by each symptom in the past month, ranging from 1 ("Not at all") to 5 ("Extremely"). Participants who presented with at least 1 intrusive symptom, 3 avoidance symptoms, and 2 hyperarousal symptoms were classified as PTSD (Weathers et al., 1994). The PCL-C for DSM-IV shows strong psychometric properties (Wilkins, Lang, & Norman, 2011). The PCL total also provides an ordinal measure of symptom severity with a recommended clinical PTSD cut-off of 50, and subclinical PTSD of 40 (National Center for Posttraumatic Stress Disorder, n.d.).

***Posttraumatic Cognitions Inventory*.** The PTCI (Foa, Ehlers, Clark, Tolin, & Orsillo, 1999) is a 33-item self-report measure of negative posttraumatic appraisals regarding the self, world, and self-blame. Responses are made on a 7-point Likert scale from 1 ("totally disagree") to 7 ("totally agree"). The PTCI has demonstrated good psychometric properties (Foa et al., 1999).

4.3.3 Fear conditioning and extinction paradigm.

The present study employed a standardized differential fear conditioning and extinction paradigm used previously (Orr et al., 2000; Zuj et al., 2016). Findings from a subset of the participants in the current study have been reported elsewhere examining the impact of hours-since-waking on fear extinction learning in PTSD (Zuj et al., 2016). The unconditioned stimulus (US) was a 500ms mild electric shock delivered to the first interosseous muscle of the dominant hand, set to a level considered "highly annoying, but not

painful" by each participant prior to the task (Orr et al., 2000). Conditioned stimuli were red and blue circles presented individually for 12s on a computer screen. The testing protocol included four experimental phases: *habituation*, *acquisition*, *early extinction*, and *late extinction*. During *habituation*, participants were exposed to four trials of each colored circle (eight trials in total). During *acquisition*, one of the colored circles (CS+) was followed by the US (mild electric shock) on all five trials (100% reinforcement schedule; Orr et al., 2000) while the other colored circle was not reinforced on any of the five trials (CS-; ten trials in total). The *early extinction* phase consisted of five trials of the CS+ (with no reinforcement) and five trials of the CS- (ten trials in total), followed by a short break of approximately one minute before participants completed the *late extinction* phase, which mirrored early extinction. Trial order was pseudo-random, with no more than two consecutive CS+ or CS- trials, and inter-trial intervals ranged from 12-21 seconds.

4.3.4 Skin conductance response.

Skin conductance level was measured through a $22mV_{rms}$, 75Hz constant-voltage coupler (FE116, ADI Instruments) with bipolar electrodes on the intermediate phalange of the first and third fingers of the non-dominant hand, sampled at 512Hz and stored at 64Hz, and recorded in micro-siemens (μS). Skin conductance response (SCR) to the CS+ and CS- was calculated by subtracting the mean SCL during the 2s prior to stimulus onset from the maximum SCL during the 12s stimulus duration. SCR values were square-root transformed, and the absolute value of negative scores was transformed and the negative sign replaced. Differential conditioned responding (DCR) was calculated by subtracting the SCR of the first CS- from the first CS+, for all trials (Menz et al., 2013; Zuj et al., 2016). For use in moderation analyses, DCR change scores were calculated for the extinction phases by subtracting the trial 5 DCR from the trial 1 DCR, with higher values representing a greater

change in differential fear responding across the experimental phase (i.e., better fear extinction performance).

4.3.5 US-expectancy ratings.

During the 12s stimulus presentation, participants were asked to rate their threat expectancy of the US on a 0-100 visual analogue scale (VAS; 0 “certain no electrical stimulus”; 100 “certain electrical stimulus”; as previously used by Lommen et al., 2013).

4.3.6 Statistical analyses.

Three (Group) × 2 (CS) × 5 (trial) mixed model analyses of variance (ANOVA) were conducted for each phase (with four trials for habituation and acquisition) to examine fear conditioning and extinction across groups. The first CS+/- trials were removed from acquisition analyses as the US had not been encountered, and no learning had occurred. Analyses were identical for both SCR and US-expectancy data, however the latter was used as a measure of contingency awareness. Greenhouse-Geisser corrections were made for within-subjects variables where necessary. Brown-Forsythe *F*-ratio corrections were made where homogeneity of variance was violated. Pairwise comparisons were conducted with Bonferroni corrections or Games-Howell tests where appropriate. Moderation analyses were conducted using the PROCESS macro for SPSS (Model 1; Hayes, 2013). An alpha level of $\alpha = .05$ was used for all tests of statistical significance. Effect sizes are reported as Cohen’s *d* following the criteria of 0.2, 0.5, and 0.8 as small, moderate and large effects, respectively (Cohen, 1988). Partial-eta squared (η_p^2) are reported as effect sizes for mixed-model ANOVAs.

4.4 Results

4.4.1 Descriptive and clinical data.

One-way ANOVA showed there was a significant between-group difference in PTSD total symptom severity, $F(2, 25.99) = 135.20$, $p < .001$, with the PTSD group displaying significantly elevated PTSD symptoms compared to TC and NTC groups. Further, the PTSD group displayed significantly higher levels of negative appraisals, and symptoms of depression, anxiety, and stress (see Table 1 for demographic and inferential data).

Table 1

Mean Scores and SDs of Demographic and Clinical Measures.

Measures	PTSD (*n* = 21)	TC (*n* = 33)	NTC (*n* = 26)	Test statistic	*p*
Demographic data					
- Age (years)	32.67 (14.62)	27.45 (10.05)	24.31 (9.82)	$F_{(2, 52.27)} = 2.90$	.064
- Sex	13F, 8M	15F, 18M	19F, 7M	$\chi^2_{(2)} = 4.70$	.096
PCL-C					
- Total	52.52 (11.38)	23.39 (3.98)	19.85 (2.57)	$F_{(2, 25.99)} = 135.20$	< .001
- Intrusive	3.00 (1.22)	0.27 (0.52)	0.00 (0.00)	$F_{(2, 77)} = 126.51$	< .001
- Avoidance	4.24 (1.81)	0.42 (0.71)	0.19 (0.49)	$F_{(2, 27.90)} = 82.61$	< .001
- Hyperarousal	3.52 (1.03)	0.39 (0.66)	0.15 (0.46)	$F_{(2, 41.75)} = 137.73$	< .001
PTCI					
- Total	113.32 (36.91)	73.33 (28.90)	61.81 (23.60)	$F_{(2, 75)} = 17.91$	< .001
- Self	2.92 (1.21)	1.83 (0.83)	1.58 (0.63)	$F_{(2, 41.12)} = 12.31$	< .001
- World	4.59 (1.33)	3.02 (1.23)	2.15 (0.82)	$F_{(2, 75)} = 25.43$	< .001
- Self-blame	3.23 (1.48)	2.16 (1.09)	2.15 (1.24)	$F_{(2, 75)} = 5.39$	.006
DASS					
- Depression	9.43 (5.76)	2.21 (2.38)	1.27 (1.76)	$F_{(2, 29.33)} = 30.64$	< .001
- Anxiety	8.14 (4.26)	1.97 (1.91)	1.00 (1.60)	$F_{(2, 32.32)} = 39.92$	< .001
- Stress	13.62 (6.34)	4.88 (3.17)	2.38 (2.23)	$F_{(2, 33.26)} = 40.83$	< .001
AUDIT	6.86 (5.46)	6.12 (3.92)	6.00 (4.14)	$F_{(2, 77)} = 0.25$	.779

Note: PCL-C = PTSD Checklist-Civilian version; PCTI= Posttraumatic Cognitions Inventory; DASS = Depression Anxiety Stress Scale; AUDIT = Alcohol Use Disorders Identification Test.

4.4.2 SCR amplitude data.

***Habituation*.** During the habituation phase, mixed-model ANOVA showed a significant main effect of trial, $F(2.87, 220.71) = 2.75$, $p = .046$, $\eta_p^2 = .034$, $\varepsilon = .955$, as SCRs reduced over the course of the phase as participants became habituated to the task (see Figure 1A).

***Acquisition*.** During the acquisition phase, mixed-model ANOVA showed a significant main effect of CS, $F(1, 77) = 81.88$, $p < .001$, $d = 0.88$, with significantly greater SCRs to the CS+ ($M = 0.86$, 95% CI[0.73, 0.99], $SD = 0.58$) than the CS- ($M = 0.39$ [0.28, 0.49], $SD = 0.49$) reflecting acquisition of fear conditioning to the CS+. There was also a significant main effect of trial, $F(2.85, 219.27) = 4.66$, $p = .004$, $\eta_p^2 = .057$, $\varepsilon = .949$, with SCRs decreasing on average across the acquisition phase (see Figure 1B). No further main effects or interactions were significant.

***Early extinction*.** Mixed-model ANOVA revealed a significant main effect of CS, $F(1, 77) = 10.78$, $p = .002$, $d = 0.31$, showing that, pooled across group and trial, the CS+ continued to elicit a greater SCR ($M = 0.60$ [0.51, 0.69], $SD = 0.41$) than the CS- ($M = 0.47$ [0.38, 0.56], $SD = 0.40$). Further, there was a significant main effect of trial, $F(3.51, 269.88) = 33.65$, $p < .001$, $\eta_p^2 = .304$, $\varepsilon = .876$, and a significant group × trial interaction, $F(7.01, 269.88) = 2.16$, $p = .038$, $\eta_p^2 = .053$, $\varepsilon = .876$. Test of simple main effects show that the TC and NTC groups demonstrate a significant reduction in SCRs from trial 1 to trial 2 ($p < .001$, and $p = .020$, respectively), however the PTSD group displayed no significant reduction in SCR from trial 1 to trial 2 ($p = .140$), however there was a significant reduction from trial 2 to trial 3 ($p = .021$). These findings suggest that the PTSD group displayed slower extinction learning than the TC and NTC groups (see Figure 2).

***Late extinction*.** Mixed-model ANOVA revealed that there was still a significant, albeit small effect between the CS+ (M = 0.50 [0.39, 0.60], SD = 0.47) and the CS- (M = 0.42 [0.33, 0.51], SD = 0.40), $F(1, 77) = 4.42$, $p = .039$, $d = 0.19$. Further, there was a significant main effect of trial, $F(3.35, 258.09) = 17.59$, $p < .001$, $\eta_p^2 = .186$, $\varepsilon = .838$, with SCRs decreasing across trials (see Figure 1D).

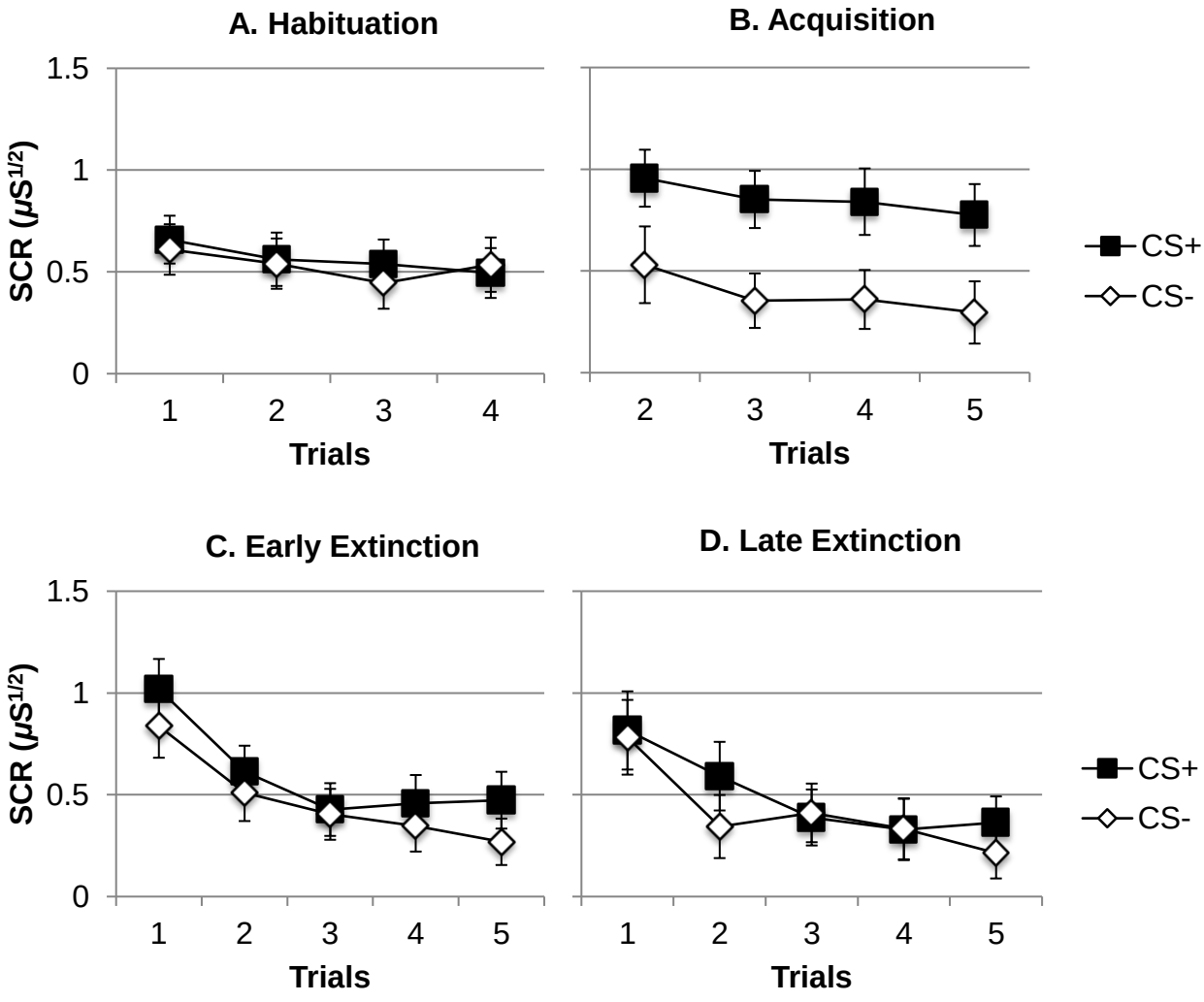

Figure 1. CS × Trial interaction for each experimental phase. Panel **(A)** shows square-root transformed SC responses during the habituation phase. Panel **(B)** shows that despite a gradual decline in SC responding, CS+/- stimulus discrimination increases across the phase.

The first CS+/- trials were removed from analyses of the acquisition phase, as the US had not been encountered, and no learning had occurred. Panels **(C)** and **(D)** show that SCRs to the CS+ and CS- decreased across the early and late extinction phases, respectively.

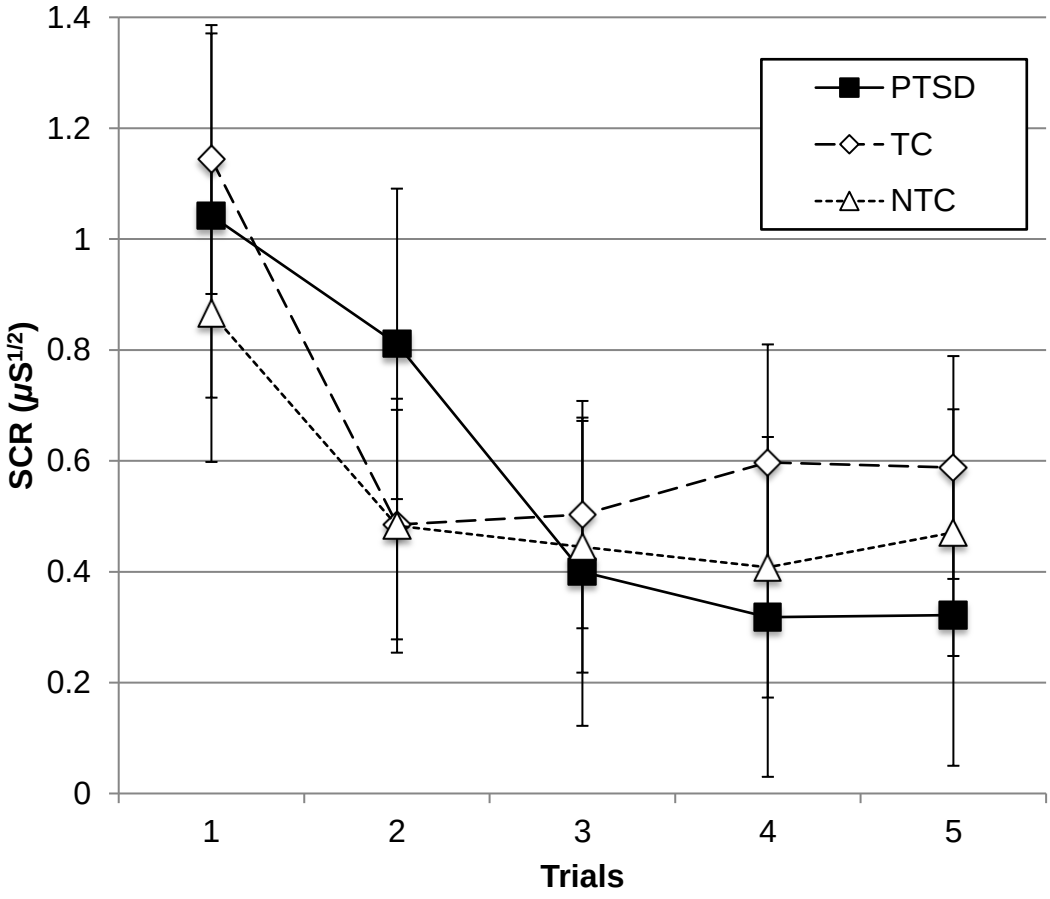

Figure 2. Group × Trial interaction during early extinction. During the early extinction phase, there was a significant group × trial interaction, showing that the PTSD group displayed a slower rate of extinction and safety signal learning across the first 3 to four trials, compared to the TC and NTC groups, who showed a rapid reduction in responding from trial 1 to trial 2. Error bars depict 95% confidence intervals of the mean. Vertical axis displays square-root transformed SCR values ($\mu S^{1/2}$).

4.4.3 Threat expectancy.

Habituation. During the habituation phase, 3 (group) × 2 (CS) × 4 (trial) mixed-model ANOVA revealed a significant main effect of trial, $F(2.02, 145.57) = 2.04$, $p = .001$, $\eta_p^2 = .092$, $\varepsilon = .674$, indicating a reduction in US-expectancy ratings over the experimental phase, pooled across CS and group.

Acquisition. During acquisition, there was a significant CS × trial interaction, $F(3.42, 242.98) = 108.76$, $p < .001$, $\eta_p^2 = .605$, $\varepsilon = .830$, with differential responding between the CS+ and CS- increasing over trials, indicating greater threat expectancy developing to the CS+ and reduced threat expectancy to the CS-.

Early extinction. There was also a significant CS × trial interaction in the early extinction phase, $F(3.93, 244.27) = 10.29$, $p < .001$, $\eta_p^2 = .125$, $\varepsilon = .619$, with differential responding decreasing over the phase. Specifically, by the end of the early extinction phase, participants are showing reduced differential threat expectancy between the CS+ and the CS-, as the CS+ ceases to predict the US (mild electric shock).

Late extinction. Further, there was also a significant CS × trial interaction in the late extinction phase and differential threat expectancy to the CS+ and CS- continued to decline, $F(3.56, 256) = 3.59$, $p = .010$, $\eta_p^2 = .048$, $\varepsilon = .558$.

4.4.4 Fear extinction and negative appraisals.

To examine the relationship between fear extinction learning and negative appraisals in PTSD, a moderation analysis was conducted (Model 1; Hayes, 2013). PCL total was included as the outcome variable, and PTCI total was included as the moderator. As primary

group effects in fear extinction learning were found during early extinction, an early extinction DCR change score was calculated and entered into the model as the predictor variable. The total model predicted a significant amount of variance in PTSD symptoms, R^2 = .450, $F(3, 74) = 20.18$, $p < .001$. As seen in Table 2, both negative appraisals and early extinction DCR change showed significant relationship with PTSD symptoms, however there was no significant interaction between negative appraisals and extinction. That is, negative appraisals did not moderate the relationship between fear extinction learning and PTSD symptom severity. Analyses were repeated with the self, world, and self-blame subscales of the PTCI as moderator variables, which yielded similar, albeit weaker patterns of effects (see supplementary materials).

Table 2

Linear model of predictors of PCL total.

	b	SE *b*	*t*	*p*
Constant	29.34 [26.82, 31.86]	1.26	23.22	< .001
Negative appraisals	0.28 [0.21, 0.36]	0.04	7.74	< .001
Early extinction DCR change	-2.62 [-5.15, -0.09]	1.27	-2.06	.043
Negative appraisals × early extinction	-0.03 [-0.11, 0.04]	0.04	-0.92	.359

Note: SE = Standard Error; Square brackets show 95% confidence intervals of *b*.

4.5 Discussion

The results of the present study reveal that both negative appraisals of the trauma and its sequelae, as well as impaired fear extinction learning are associated with elevated PTSD

symptoms. This study was the first to our knowledge to examine whether negative appraisals and fear extinction learning interact to influence PTSD symptoms. Moderation analyses revealed that negative appraisals did not moderate the relationship between fear extinction and PTSD symptoms, suggesting that fear extinction and negative appraisals do not interact in PTSD. This provides novel evidence that negative appraisals and fear extinction play independent roles in PTSD symptoms.

Ehlers and Clark (2000) proposed that a key mechanisms underlying the development of PTSD is excessive negative appraisals of the trauma and its sequelae (also see Ehlers et al., 2003). Using the PTCI as an index of trauma-related negative appraisals, our results confirm this prediction by finding that the PTCI total has a significant positive relationship with PTSD symptoms. This supports a wealth of prior studies indicating the importance of excessively negative appraisals in PTSD (e.g., Dunmore et al., 1999, 2001; Ehlers et al., 1998). Analyses with the PTCI self, world, and self-blame subscales showed similar patterns with the PTCI total score (see Supplementary Materials). Participants in the current study experienced a range of interpersonal and disaster-related traumas, with many participants experiencing both. Future studies examining specific trauma populations may shed light on the contributions of individual trauma-types on negative appraisals and PTSD symptoms.

There is considerable evidence of PTSD-related impairments in fear extinction learning (Norrholm et al., 2011; Orr et al., 2000; Peri, Ben-Shakhar, Orr, & Shalev, 2000; Zuj et al., 2016), and the findings of the current study support this notion. Specifically, we found that the PTSD group displayed a slower reduction in conditioned responding during the early extinction phase, which is concordant with the idea of fear load (Norrholm et al., 2015). That is, PTSD is associated with greater expression of fear during the early stages of extinction learning, relative to comparison groups. Further, rapid fear extinction may be due in part to the 100% reinforcement schedule used during fear acquisition, which may also explain the

trial main effect found during acquisition, where SCRs gradually declined across the experimental phase as participants habituated to the task. Studies failing to find group differences in extinction learning have found PTSD to be associated with impaired recall of fear extinction learning 24h later, compared to controls (e.g., Milad et al., 2008; Milad et al., 2009). Future research should employ 2-day extinction learning and recall designs to assess the interactive effects of negative appraisals and fear extinction on the recall of extinction learning.

The findings of the present study have important potential implications for treatment strategies. Previous research has found comparable treatment gains between exposure therapy alone, cognitive restructuring alone, or a combination of the two (Marks, Lovell, Noshirvani, Livanou, & Thrasher, 1998; Tarrier, Sommerfield, Pilgrim, & Humphreys, 1999). Bryant, Moulds, Guthrie, Dang, and Nixon (2003), however, found that participants who underwent imaginal exposure combined with cognitive restructuring showed a greater reduction of PTSD symptoms than imaginal exposure alone. The findings of the current study suggest that both cognitive therapy for negative appraisals and exposure therapy for fear extinction are important elements to be included in treatment, as both have independent effects on PTSD symptoms (e.g., Marks et al., 1998).

The cognitive model further hypothesizes that persistent PTSD is caused by fragmented and poorly contextualized trauma memories, and dysfunctional behavioral and cognitive strategies aimed at reducing feelings of threat, yet further exaggerating the problem (Ehlers et al., 2003). A limitation of the current study is that we were unable to examine these aspects of the model, and future research would benefit from investigating the role of extinction in the elaboration of trauma memories, and the nature of intrusive memories in PTSD. Specifically, extinction networks show an important role of the basolateral amygdala in the expression or inhibition of fear (Milad & Quirk, 2012), and this neural substrate is also

involved in emotional memory consolidation (e.g., Roozendaal et al., 2009; Roozendaal, Schelling, & McGaugh, 2008). This suggests a neurobiological basis for impaired extinction memory as a mechanism in the intrusive re-experiencing symptoms of PTSD. As fear extinction recall impairments are a robust finding in PTSD (e.g., Milad et al., 2008; Milad et al., 2009), specifically assessing the memory for fear extinction may show stronger convergences between extinction and cognitive models of PTSD.

The present study had some limitations, including a relatively modest clinical sample, the use of a cross-sectional design, and the lack of investigation of fear extinction recall measures, which have been one of the most robust findings in terms of potential mechanisms in PTSD. To further delineate the role of these potential mechanisms, future research is needed that employs longitudinal prospective designs to test the independent and interactive effects of negative appraisals and fear extinction learning (and recall of fear extinction learning) on PTSD symptom development over time. Employing a partial reinforcement schedule may test whether these relationships stand under conditions of less predictable threat, which may reduce the speed of extinction learning and enhance sensitivity of this measure.

In conclusion, as far as we are aware this is the first study to examine the interactive effects of negative, trauma-related appraisals and impaired fear extinction learning on PTSD symptomatology. We found evidence for an independent effect of both negative appraisals and fear extinction learning on PTSD symptoms. This supports the two key proposed mechanisms in a prevailing cognitive model of PTSD (Ehlers and Clark, 2000), but we did not find an interaction between cognitions and fear extinction learning. This highlights there are both cognitive and biological processes involved in PTSD, and both require addressing in treatment. In an experimental setting, we show that negative appraisals and fear extinction

function independently in PTSD, and treatment may be optimized by targeting both of these factors.

4.6 References

American Psychiatric Association. (2000). *Diagnostic and Statistical Manual of Mental Disorders* (4th ed., text revision ed.). Washington, DC: American Psychiatric Association.

Atwoli, L., Stein, D. J., Koenen, K. C., & McLaughlin, K. A. (2015). Epidemiology of posttraumatic stress disorder: prevalence, correlates and consequences. *Current Opinion in Psychiatry, 28*(4), 307-311. doi:10.1097/yco.0000000000000167

Bryant, R. A., & Guthrie, R. M. (2007). Maladaptive self-appraisals before trauma exposure predict posttraumatic stress disorder. *Journal of Consulting and Clinical Psychology, 75*(5), 812-815. doi:10.1037/0022-006x.75.5.812

Bryant, R. A., Moulds, M. L., Guthrie, R. M., Dang, S. T., & Nixon, R. D. (2003). Imaginal exposure alone and imaginal exposure with cognitive restructuring in treatment of posttraumatic stress disorder. *Journal of Consulting and Clinican Psychology, 71*(4), 706-712.

Cohen, J. (1988). *Statistical power analysis for the behavioural sciences* (2nd ed.). Hillsdale, NJ: Lawrence Erlbaum.

Davis, M., & Myers, K. M. (2002). The role of glutamate and gamma-aminobutyric acid in fear extinction: clinical implications for exposure therapy. *Biological Psychiatry, 52*(10), 998-1007.

Dunmore, E., Clark, D. M., & Ehlers, A. (1999). Cognitive factors involved in the onset and maintenance of posttraumatic stress disorder (PTSD) after physical or sexual assault. *Behaviour Research and Therapy, 37*, 809-829.

Dunmore, E., Clark, D. M., & Ehlers, A. (2001). A prospective investigation of the role of cognitive factors in persistent posttraumatic stress disorder (PTSD) after physical or sexual assault. *Behaviour Research and Therapy, 39*, 1063-1084.

Ehlers, A., & Clark, D. M. (2000). A cognitive model of posttraumatic stress disorder. *Behaviour Research and Therapy, 38*, 319-345.

Ehlers, A., Mayou, R. A., & Bryant, B. (1998). Psychological predictors of chronic posttraumatic stress disorder after motor vehicle accidents. *Journal of Abnormal Psychology, 107*(3), 508-519.

Ehlers, A., Mayou, R. A., & Bryant, B. (2003). Cognitive predictors of posttraumatic stress disorder in children: results of a prospective longitudinal study. *Behaviour Research and Therapy, 41*(1), 1-10.

Ehring, T., Ehlers, A., & Glucksman, E. (2008). Do cognitive models help in predicting the severity of posttraumatic stress disorder, phobia, and depression after motor vehicle accidents? A prospective longitudinal study. *Journal of Consulting and Clinical Psychology, 76*(2), 219-230. doi:10.1037/0022-006x.76.2.219.supp

Foa, E. B., Ehlers, A., Clark, D. M., Tolin, D. F., & Orsillo, S. M. (1999). The Posttraumatic Cognitions Inventory (PTCI): Development and validation. *Psychological Assessment, 11*(3), 303-314. doi:10.1037/1040-3590.11.3.303

Guthrie, R. M., & Bryant, R. A. (2006). Extinction learning before trauma and subsequent posttraumatic stress. *Psychosomatic Medicine, 68*, 307-311. doi:10.1097/01.psy.0000208629.67653.cc

Hayes, A. F. (2013). *Introduction to mediation, moderation, and conditional process analysis: A regression-based approach*. New York: The Guilford Press.

Lommen, M. J. J., Engelhard, I. M., Sijbrandij, M., van den Hout, M. A., & Hermans, D. (2013). Pre-trauma individual differences in extinction learning predict posttraumatic stress. *Behaviour Research and Therapy, 51*(2), 63-67. doi:10.1016/j.brat.2012.11.004

Marks, I., Lovell, K., Noshirvani, H., Livanou, M., & Thrasher, S. (1998). Treatment of posttraumatic stress disorder by exposure and/or cognitive restructuring: a controlled study. *Archives of General Psychiatry, 55*(4), 317-325.

Menz, M. M., Rihm, J. S., Salari, N., Born, J., Kalisch, R., Pape, H. C., . . . Buchel, C. (2013). The role of sleep and sleep deprivation in consolidating fear memories. *Neuroimage, 75*, 87-96. doi:10.1016/j.neuroimage.2013.03.001

Milad, M. R., Orr, S. P., Lasko, N. B., Chang, Y., Rauch, S. L., & Pitman, R. K. (2008). Presence and acquired origin of reduced recall for fear extinction in PTSD: Results of a twin study. *Journal of Psychiatric Research, 42*(7), 515-520. doi:10.1016/j.jpsychires.2008.01.017

Milad, M. R., Pitman, R. K., Ellis, C. B., Gold, A. L., Shin, L. M., Lasko, N. B., . . . Rauch, S. L. (2009). Neurobiological basis of failure to recall extinction memory in posttraumatic stress disorder. *Biological Psychiatry, 66*(12), 1075-1082. doi:10.1016/j.biopsych.2009.06.026

Milad, M. R., & Quirk, G. J. (2012). Fear extinction as a model for translational neuroscience: Ten years of progress. In S. T. Fiske, D. L. Schacter, & S. E. Taylor (Eds.), *Annual Review of Psychology* (Vol. 63, pp. 129-151).

National Center for Posttraumatic Stress Disorder. (n.d.). Using the PTSD Checklist for DSM-IV (PCL). Retrieved from http://www.ptsd.va.gov/professional/pages/assessments/assessment-pdf/PCL-handout.pdf

Norrholm, S. D., Glover, E. M., Stevens, J. S., Fani, N., Galatzer-Levy, I. R., Bradley, B., . . . Jovanovic, T. (2015). Fear load: The psychophysiological over-expression of fear as an intermediate phenotype associated with trauma reactions. *International Journal of Psychophysiology, 98*(2 Pt 2), 270-275. doi:10.1016/j.ijpsycho.2014.11.005

Norrholm, S. D., Jovanovic, T., Olin, I. W., Sands, L. A., Karapanou, I., Bradley, B., & Ressler, K. J. (2011). Fear extinction in traumatized civilians with posttraumatic stress disorder: Relation to symptom severity. *Biological Psychiatry, 69*(6), 556-563. doi:10.1016/j.biopsych.2010.09.013

Orr, S. P., Lasko, N. B., Macklin, M. L., Pineles, S. L., Chang, Y., & Pitman, R. K. (2012). Predicting post-trauma stress symptoms from pre-trauma psychophysiologic reactivity, personality traits and measures of psychopathology. *Biology of Mood & Anxiety Disorders, 2*(8).

Orr, S. P., Metzger, L. J., Lasko, N. B., Macklin, M. L., Peri, T., & Pitman, R. K. (2000). De novo conditioning in trauma-exposed individuals with and without posttraumatic stress disorder. *Journal of Abnormal Psychology, 109*(2), 290-298. doi:10.1037/0021-843x.109.2.290

Peri, T., Ben-Shakhar, G., Orr, S. P., & Shalev, A. Y. (2000). Psychophysiologic assessment of aversive conditioning in posttraumatic stress disorder. *Biological Psychiatry, 47*(6), 512-519. doi:10.1016/s0006-3223(99)00144-4

Pitman, R. K., Rasmusson, A. M., Koenen, K. C., Shin, L. M., Orr, S. P., Gilbertson, M. W., . . . Liberzon, I. (2012). Biological studies of post-traumatic stress disorder. *Nature, 13*, 769-787. doi:10.1038/nm3339

Ramchand, R., Schell, T. L., Karney, B. R., Osilla, K. C., Burns, R. M., & Caldarone, L. B. (2010). Disparate prevalence estimates of PTSD among service members who served in Iraq and Afghanistan: possible explanations. *Journal of Traumatic Stress, 23*(1), 59-68. doi:10.1002/jts.20486

Roozendaal, B., McReynolds, J. R., Van der Zee, E. A., Lee, S., McGaugh, J. L., & McIntyre, C. K. (2009). Glucocorticoid effects on memory consolidation depend on functional interactions between the medial prefrontal cortex and basolateral amygdala. *Journal of Neuroscience, 29*(45), 14299-14308. doi:10.1523/jneurosci.3626-09.2009

Roozendaal, B., Schelling, G., & McGaugh, J. L. (2008). Corticotropin-releasing factor in the basolateral amygdala enhances memory consolidation via an interaction with the beta-adrenoceptor-cAMP pathway: Dependence on glucocorticoid receptor activation. *Journal of Neuroscience, 28*(26), 6642-6651. doi:10.1523/jneurosci.1336-08.2008

Shvil, E., Sullivan, G. M., Schafer, S., Markowitz, J. C., Campeas, M., Wager, T. D., . . . Neria, Y. (2014). Sex differences in extinction recall in posttraumatic stress disorder: A pilot fMRI study. *Neurobiology of Learning and Memory*. doi:10.1016/j.nlm.2014.02.003

Tarrier, N., Sommerfield, C., Pilgrim, H., & Humphreys, L. (1999). Cognitive therapy or imaginal exposure in the treatment of post-traumatic stress disorder. Twelve-month follow-up. *British Journal of Psychiatry, 175*, 571-575.

Vrana, S. R., & Lauterbach, D. (1994). Prevalence of traumatic events and post-traumatic psychological symptoms in a nonclinical sample of college students. *Journal of Traumatic Stress, 7*, 289-302.

Weathers, F. W., Litz, B. T., Huska, J. A., & Keane, T. M. (1994). *The PTSD Checklist-Civilian Version (PCL-C) for DSM-IV*. Boston: National Center for PTSD, Behavioral Sciences Division.

Wilkins, K. C., Lang, A. J., & Norman, S. B. (2011). Synthesis of the psychometric properties of the PTSD checklist (PCL) military, civilian, and specific versions. *Depression and Anxiety, 28*(7), 596-606. doi:10.1002/da.20837

Zuj, D. V., Palmer, M. A., Hsu, C. K., Nicholson, E. L., Cushing, P. J., Gray, K. E., & Felmingham, K. L. (2016). Impaired fear extinction associated with PTSD increases with hours-since-waking. *Depression and Anxiety, 33*(3), 203-210. doi:10.1002/da.22463

4.7 Supplementary Material

Table S1. Moderation analysis with PTCI self subscale as the moderator

Table S2. Moderation analysis with PTCI world subscale as the moderator

Table S3. Moderation analysis with PTCI self-blame subscale as the moderator

Table S1

Moderation Analysis with PTCI Self Subscale as the Moderator.

	b	SE *b*	*t*	*p*
Constant	29.46 [26.90, 32.02]	1.29	22.93	< .001
Negative appraisals (self)	9.80 [7.15, 12.46]	1.33	7.36	< .001
Early extinction DCR change	-2.23 [-4.81, 0.36]	1.30	-1.72	.090
Negative appraisals × early extinction	-2.03 [-4.60, 0.54]	1.29	-1.58	.537

Note: SE = standard error. Square brackets show 95% confidence intervals of *b*.

Table S2

Moderation Analysis with PTCI World Subscale as the Moderator.

	b	SE *b*	*t*	*p*
Constant	29.12 [26.46, 31.79]	1.34	21.78	< .001
Negative appraisals (world)	6.16 [4.32, 8.01]	0.92	6.67	< .001
Early extinction DCR change	-2.25 [-4.95, 0.45]	1.36	-1.66	.101
Negative appraisals × early extinction	-0.03 [-1.97, 1.92]	0.98	-0.03	.980

Note: SE = standard error. Square brackets show 95% confidence intervals of *b*.

Table S3

Moderation Analysis with PTCI Self-blame Subscale as the Moderator.

	b	SE *b*	*t*	*p*
Constant	29.09 [26.09, 32.08]	1.50	19.33	< .001
Negative appraisals (self-blame)	4.15 [1.81, 6.49]	1.18	3.53	< .001
Early extinction DCR change	-0.91 [-3.93, 2.12]	1.52	-0.60	.552
Negative appraisals × early extinction	-2.09 [-4.93, 0.76]	1.43	-1.46	.148

Note: SE = standard error. Square brackets show 95% confidence intervals of *b*.

Chapter 5

Endogenous Noradrenaline does not Interact with PTSD-Related Fear Extinction Learning Impairments

This chapter is currently in preparation for submission for peer-review:

Zuj, D. V., Palmer, M. A., Malhi, G. S., Bryant, R. A., & Felmingham, K. L. Endogenous noradrenaline does not interact with PTSD-related fear extinction learning impairments.

5.1 Abstract

Background: Posttraumatic stress disorder (PTSD) is associated with hyperactive noradrenergic signaling, and learning and memory performance is enhanced with increased noradrenaline (NA) activity. Learning to extinguish emotional fear reactions to trauma-related triggers is the goal of exposure-based therapies, and is likely to involve key noradrenergic processes, however the relationship between endogenous NA activity and fear extinction learning ability in PTSD is largely unknown.

Method: Here, a sample of participants with PTSD (n = 21), trauma exposure without PTSD (n = 36), and non-trauma-exposed controls (n = 27) completed a standardized differential fear conditioning and extinction paradigm, and provided saliva samples for salivary alpha-amylase (as an index of NA levels). Skin conductance response (SCR) served as the index of conditioned responding, and the unconditioned stimulus (US) was a 500ms mild electric shock.

Results: There were no significant between-group differences on baseline or post-acquisition NA levels. There was a PTSD-specific impairment in fear extinction learning during the early extinction phase where the PTSD group displayed a slower reduction of SC responses compared to the TC and NTC groups. A moderation analysis revealed that NA did not moderate the relationship between early extinction learning and PTSD symptoms.

Conclusions: These findings suggest that the relationship between fear extinction learning ability and PTSD symptoms does not change as a function of salivary NA levels. As elevated NA levels are thought to facilitate both learning and memory processes, future research should replicate the current study with a 2-day fear extinction recall paradigm to investigate the role of NA in the memory for fear extinction.

5.2 Introduction

Hyperactive noradrenergic signaling is considered a hallmark correlate of posttraumatic stress disorder (Zoladz & Diamond, 2013), a chronic psychiatric condition that can develop following a severe traumatic event (American Psychiatric Association, 2013). Elevated noradrenergic signaling of stress hormones such as noradrenaline (NA) is associated with enhanced emotional memory formation (Mueller & Cahill, 2010). The aim of exposure-based therapies for PTSD is to facilitate the extinction of conditioned fear memories associated with triggers for the trauma memory. Behavioral research in healthy controls suggest that strategically increasing NA release at key points of a fear conditioning and extinction paradigm can strengthen conditioned fear (Antov, Wolk, & Stockhorst, 2013), or enhance the consolidation of extinction memories (Antov, Melicherová, & Stockhorst, 2015). These findings indicate that NA levels may interact with fear extinction learning performance in PTSD, with important implications for exposure-based therapies.

During situations of heightened arousal such as trauma and stress, the sympathetic nervous system (SNS) signals fast NA release (among other catecholamines and neurotransmitters; Southwick et al., 1999). Consistent evidence shows that clinical patients and combat veterans with PTSD display elevated baseline NA levels (Geracioti et al., 2001; Glover & Poland, 2002; Lemieux & Coe, 1995; Yehuda et al., 1998; Young & Breslau, 2004). Elevated baseline NA in PTSD is aligned with the hypothesis of the SNS contributing to increased situational arousal and re-experiencing symptoms of PTSD (Griffin, Resick, & Galovski, 2012; Liberzon, Abelson, Flagel, Raz, & Young, 1999). Furthermore, greater NA levels in combat veterans within two months of deployment predicted increased PTSD symptom severity three months later (Highland et al., 2015), suggesting that heightened noradrenergic signaling strengthened conditioned fear associations. An additional longitudinal study, however, found that peripheral NA levels shortly post-trauma were a poor

risk factor for PTSD in civilian trauma victims (Videlock et al., 2008), suggesting that other factors may be involved in the relationship between hyperactive noradrenergic signaling and PTSD symptoms.

Enhanced NA activity is implicated in stronger emotional memory formation (Mueller & Cahill, 2010), and a prevailing model of PTSD posits that benign stimuli present at the time of the trauma act as triggers for conditioned emotional fear memories (e.g., Mineka & Oehlberg, 2008; Pitman et al., 2012). Ample evidence demonstrates that individuals with PTSD show impaired extinction of these conditioned fear associations (Blechert, Michael, Vriends, Margraf, & Wilhelm, 2007; Norrholm et al., 2011; Orr et al., 2000; Peri, Ben-Shakhar, Orr, & Shalev, 2000; Zuj et al., 2016). Indeed, successful fear extinction is the aim of exposure-based therapies for PTSD and other fear-related disorders, and therefore greater understanding of the relationship between NA activity and fear extinction carries important implications for improving current treatment methods. Recently, Soeter and Kindt (2012) administered yohimbine (leading to increased NA release) to healthy individuals immediately prior to fear acquisition, resulting in a conditioned memory trace that was broadly generalized and resistant to extinction learning. Similarly, a study using a cold pressor test in healthy males to induce a noradrenergic stress reaction found that the cold pressor test immediately prior to fear acquisition resulted in a conditioned fear memory trace that was resistant to extinction (Antov et al., 2013). Alternatively, the cold pressor test used immediately before extinction learning resulted in enhanced extinction learning (Antov et al., 2015). These findings suggest that noradrenergic signaling may shape fear conditioning and extinction learning, however this has not been tested in patients with PTSD.

The current study used a standardized differential fear conditioning and extinction paradigm to investigate the role of endogenous salivary NA activity in fear extinction learning and PTSD, compared to trauma-exposed and non-exposed control groups without

PTSD. Based on previous research using behavioral tasks to manipulate NA levels in healthy participants (e.g., Antov et al., 2015; Antov et al., 2013), we predicted that endogenous NA levels would moderate the relationship between fear extinction learning ability and PTSD symptoms. Specifically, PTSD is consistently associated with impaired fear extinction learning (e.g., Norrholm et al., 2011; Zuj et al., 2016), however this relationship may become stronger with lower NA activity.

5.3 Method

5.3.1 Participants.

Eighty-four participants aged 18-63 years (M = 27.6 years, SD = 11.4) and comprising 37 males and 47 females were involved in the study. Participants were allocated to groups on the basis of exposure to a criterion A stressor (American Psychiatric Association, 2013) using the Traumatic Events Questionnaire (TEQ; Vrana & Lauterbach, 1994). The PTSD Checklist-Civilian version (PCL-C; Weathers, Litz, Huska, & Keane, 1994) was used to estimate PTSD symptom severity – participants who displayed at least 1 intrusive symptom, 3 avoidance symptoms, and 2 hyperarousal symptoms were classified as PTSD (Weathers et al., 1994). Participants who displayed relatively low PCL scores were classified as trauma-exposed controls (TC). Participants who reported no experience of a traumatic event were classified as non-trauma exposed controls (NTC). These criteria resulted in three groups: PTSD (n = 21), TC (n = 36), and NTC (n = 27). Participants in the PTSD and TC groups were exposed to a variety of environmental and interpersonal traumas, including war exposure (n = 5), accident (n = 21), natural disaster (n = 27), witness to serious injury or death (n = 36), assaulted or molested (n = 22), threatened or held captive (n = 12), and tortured or the victim of terrorism (n = 2). Mean years since trauma for the PTSD group was 10.1 years (SD = 12.8 years), and 10.1 years (SD = 10.9 years) for the TC group. Participants

also completed the Depression Anxiety Stress Scale – 21 item version (DASS; Lovibond & Lovibond, 1995). This study was approved by the University of Tasmania Social Science Human Research Ethics Committee, and all participants gave full informed consent.

5.3.2 Fear conditioning and extinction paradigm.

The present study employed a differential fear conditioning and extinction paradigm used previously (Orr et al., 2000; Zuj et al., 2016). Findings from a subset of participants in the current study have been reported elsewhere (Zuj et al., 2016). The unconditioned stimulus (US) was a 500ms mild electric shock delivered to the first interroseous muscle of the dominant hand, and set to a level considered "highly annoying, but not painful" by each participant prior to the task (Orr et al., 2000; Zuj et al., 2016). Red and blue circles were used as conditioned stimuli (CS), and were presented individuals for 12 seconds on a computer screen. The testing protocol included four experimental phases: *habituation*, *acquisition*, *early extinction*, and *late extinction*. During *habituation*, participants were exposed to four trials of each coloured circle (eight trials in total). During *acquisition*, one of the coloured circles (CS+) was followed by the US on all five trials (100% reinforcement schedule) while the other coloured circle was not reinforced on any of the five trials (CS-; ten trials in total). The *early extinction* phase consisted of five trials of the CS+ (with no reinforcement) and five trials of the CS- (ten trials in total). Following a short rest period of approximately one minute (Milad, Orr, Pitman, & Rauch, 2005), participants completed the *late extinction* phase, also with no presentation of the US. Trial order was pseudo-random, with no more than two consecutive CS+ or CS- trials.

5.3.3 Skin conductance response.

Skin conductance level was measured through a 22mV$_{rms}$, 75Hz constant-voltage coupler (FE116, ADInstruments) with bipolar electrodes on the intermediate phalange of the first and third fingers of the non-dominant hand, sampled at 512Hz and stored at 64Hz, and recorded in micro-Siemens (μS). Skin conductance response (SCR) to the CS+ and CS- was calculated by subtracting the mean SCL during the 2s prior to stimulus onset from the maximum SCL during the 12s stimulus duration. SCR values were square-root transformed, and the absolute value of negative scores was transformed and the negative sign replaced. Differential conditioned responding (DCR) was calculated by subtracting the SCR of the first CS- from the first CS+, for all trials (Menz et al., 2013). For use in moderation analyses, DCR change scores were calculated for the extinction phases by subtracting the trial 5 DCR from the trial 1 DCR, with higher values representing a greater change in differential fear responding across the experimental phase (i.e., better fear extinction performance).

5.3.4 US-expectancy ratings.

During the 12s stimulus presentation, participants were asked to rate their threat expectancy of the US on a 0-100 visual analogue scale (VAS; 0 “certain no electrical stimulus”; 100 “certain electrical stimulus”; as previously used by Lommen, Engelhard, Sijbrandij, van den Hout, & Hermans, 2013).

5.3.5 Salivary alpha-amylase.

All participants provided saliva samples at the beginning of testing, and immediately post-fear acquisition. Endogenous noradrenergic activity was indexed via salivary alpha-amylase (sAA), which has received support from human and animal research as a valid biomarker of noradrenergic activity (Nater & Rohleder, 2009). Samples were collected

immediately post-fear acquisition as NA has an immediate peak latency response post-threat (Nater & Rohleder, 2009). As sAA is an enzyme found in saliva, participants were requested to refrain from exposure to cigarettes, food, or liquids (other than water) for one hour prior to the study (Rohleder & Nater, 2009). Samples were analyzed using a commercially available ELISA assay (Salimetrics, USA) according to the manufacturers instructions, with intra- and inter-assay variability of 5.6% and 6.3%, respectively. Baseline and post-acquisition sAA were square-root transformed to normalize distributions. sAA reactivity scores were calculated by subtracting baseline sAA levels from the post-acquisition sAA level.

5.3.6 Statistical analyses.

Three participants were excluded from all analyses due to not learning the CS-US contingency by the end of acquisition. Separate 3 (group) × 2 (CS) × 5 (trial) mixed-model ANOVAs were conducted separately for each phase (with four trials for habituation and acquisition). The first CS+/- trials were removed from acquisition analyses as the US had not been encountered, and no learning had occurred. Greenhouse-Geisser corrections were made for within-subjects variables where necessary. Brown-Forsythe *F*-ratio adjustments were made where necessary, and pairwise comparisons were conducted with Bonferroni corrections or Games-Howell tests where appropriate. Moderation analyses were conducted using the PROCESS macro for SPSS (Model 1; Hayes, 2013). An alpha level of $\alpha = .05$ was used for all tests of statistical significance. Effect sizes are reported as Cohen's *d* following the criteria of 0.2, 0.5, and 0.8 as small, moderate and large effects, respectively (Cohen, 1988). Partial-eta squared (η_p^2) are reported for mixed-model ANOVAs.

5.4 Results

5.4.1 Descriptive and clinical data.

Descriptive statistics and additional inferential data are displayed in Table 1. One-way ANOVA revealed no significant between-group difference on age, $F(2, 50.76) = 2.93$, $p = .062$. As expected, there were significant between group differences in PCL total scores, $F(2, 25.55) = 135.50$, $p < .001$, with the PTSD group having significantly higher mean PTSD symptom severity than the TC and NTC groups (ps $< .001$), who also significantly differed ($p < .001$).

Table 1

Mean Scores and SDs of Demographic, Clinical and Salivary Measures.

Measures	PTSD (*n* = 21)	TC (*n* = 36)	NTC (*n* = 27)	Test statistic	*p*
Demographic data					
- Age (years)	32.67 (14.62)	26.97 (9.76)	24.48 (9.67)	$F_{(2, 50.76)} = 2.93$	.062
- Sex	13F, 8M	15F, 21M	19F, 8M	$\chi^2_{(2)} = 5.56$	.062
PCL-C					
- Total	52.52 (11.38)	23.47 (3.87)	19.93 (2.56)	$F_{(2, 25.55)} = 135.50$	< .001
- Intrusive	3.00 (1.22)	0.28 (0.51)	0.00 (0.00)	$F_{(2, 81)} = 132.52$	< .001
- Avoidance	4.24 (1.81)	0.39 (0.69)	0.19 (0.48)	$F_{(2, 27.28)} = 84.48$	< .001
- Hyperarousal	3.52 (1.03)	0.44 (0.77)	0.15 (0.46)	$F_{(2, 45.66)} = 127.70$	< .001
DASS					
- Depression	9.43 (5.76)	2.28 (2.37)	1.52 (2.15)	$F_{(2, 30.88)} = 28.47$	< .001
- Anxiety	8.14 (4.26)	1.94 (1.87)	1.11 (1.67)	$F_{(2, 32.19)} = 39.33$	< .001
- Stress	13.62 (6.34)	4.89 (3.07)	2.56 (2.36)	$F_{(2, 32.98)} = 40.04$	< .001
AUDIT	6.86 (5.46)	6.22 (3.83)	6.11 (4.10)	$F_{(2, 55.50)} = 0.18$	.837

Note: PTSD = Posttraumatic Stress Disorder; TC = Trauma-exposed control group; NTC = Non-trauma exposed control group; PCL-C = PTSD Checklist-Civilian version; DASS = Depression Anxiety Stress Scale; AUDIT = Alcohol Use Disorders Identification Test.

5.4.2 Salivary alpha-amylase.

A one-way ANOVA revealed no significant between-group differences on baseline sAA levels, $F(2, 81) = 0.05$, $p = .955$, or post-acquisition levels, $F(2, 81) = 1.04$, $p = .359$. A repeated measures ANOVA revealed there was a significant increase in sAA from baseline to post-acquisition, $F(1, 81) = 4.64$, $p = .034$, $\eta_p^2 = .054$, however there was no group × time interaction, $F(2, 81) = 2.18$, $p = .120$, $\eta_p^2 = .051$. Figure 1 displays mean sAA levels during baseline and post-acquisition.

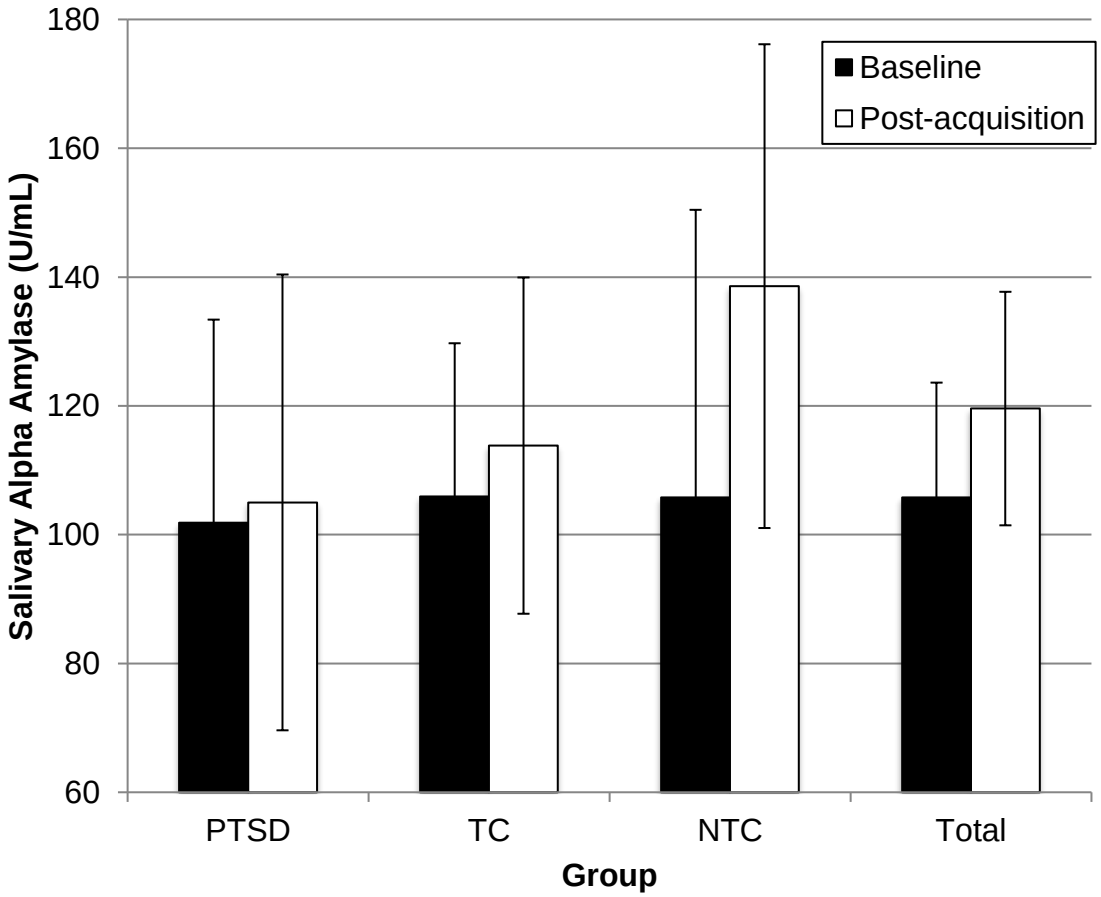

Figure 1. Group and total levels of baseline and post-acquisition salivary Alpha Amylase (sAA). On average, all groups showed an increase in sAA levels from baseline to post-

acquisition, however these levels did not differ significantly between groups. Figure shows raw sAA values. Error bars represent 95% CIs.

5.4.3 SCR amplitude data.

Habituation. Mixed-model ANOVA revealed a significant main effect of trial, $F(2.89, 233.80) = 3.08$, $p = .030$, $\eta_p^2 = .037$, $\varepsilon = .962$, with SCRs decreasing over the four trials, pooled across groups (see Figure 2A). No further main effects of interactions were significant.

Acquisition. There was a significant CS main effect, $F(1, 81) = 84.10$, $p < .001$, $d = 0.88$, with the CS+ eliciting, on average, a significantly larger SCR ($M = 0.86$, 95% CI[0.73, 0.91], $SD = 0.58$) than the CS- ($M = 0.39$ [0.29, 0.50], $SD = 0.49$). Further, there was a significant main effect of trial, $F(2.85, 230.74) = 4.58$, $p = .005$, $\eta_p^2 = .054$, $\varepsilon = .950$. Importantly, there was a significant group × CS interaction, $F(2, 81) = 3.37$, $p = .039$, $\eta_p^2 = .077$. Tests of simple effects revealed no between-group simple main effect for the CS+, $F(2, 81) = 0.15$, $p = .863$, $\eta_p^2 = .004$, however there was a marginally significant between-group effect in responding to the CS-, $F(2, 81) = 3.08$, $p = .051$, $\eta_p^2 = .071$. This effect shows that there were no significant differences between the PTSD and TC groups in responses to the CS- ($p = .734$), however both the PTSD and TC group displayed significantly greater responding to the CS- than the NTC group ($p = .032$, and $p = .037$, respectively).

Early extinction. There was a significant CS main effect, $F(1, 81) = 8.38$, $p = .005$, $d = 0.27$, with the CS+ eliciting, on average, a significantly larger SCR ($M = 0.60$ [0.51, 0.69], $SD = 0.41$) than the CS- ($M = 0.49$ [0.40, 0.58], $SD = 0.42$). Further, there was a significant

main effect of trial, $F(3.63, 293.71) = 31.98$, $p < .001$, $\eta_p^2 = .283$, $\varepsilon = .907$, and a significant group × trial interaction, $F(7.25, 293.71) = 2.19$, $p = .033$, $\eta_p^2 = .051$, $\varepsilon = .907$. Tests of simple effects show the TC and NTC groups demonstrate significant reduction in SCRs from trial 1 to trial 2 ($p < .001$, and $p = .003$, respectively) and all further between-trial changes were non-significant. The PTSD group, however, showed a significant reduction in SCRs from trial 1 to trial 2 ($p = .021$) and from trial 2 to trial 3 ($p = .002$), with no significant between-trial changes after trial 3. These findings suggest that extinction learning is slower for the PTSD group, compared to TC and NTC groups (see Figure 3).

Late extinction. ANOVA revealed a significant main effect of CS, $F(1, 81) = 4.77$, $p = .032$, $d = 0.19$, with the CS+ still eliciting greater SCRs ($M = 0.51$ [0.40, 0.61], $SD = 0.48$) than the CS- ($M = 0.42$ [0.34, 0.51], $SD = 0.40$). Further, there was a significant main effect of trial, $F(3.34, 270.51) = 17.21$, $p < .001$, $\eta_p^2 = .175$, $\varepsilon = .835$, with SCRs reducing across the phase, pooled across group and CS (see Figure 2D). No main effects or interactions involving group were significant during the late extinction phase.

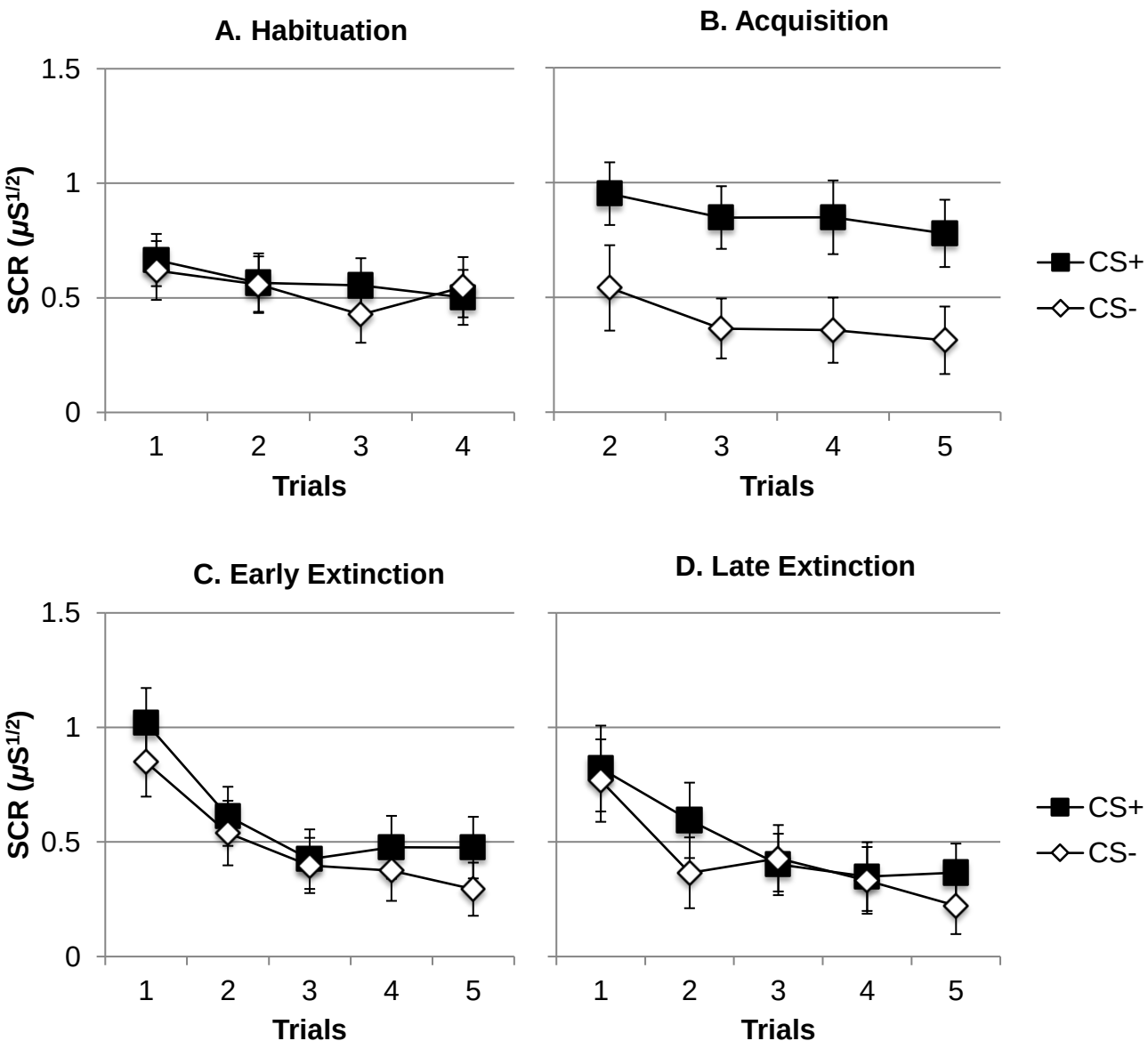

Figure 2. CS × Trial interaction for each experimental phase. Panel **(A)** shows square-root transformed SC responses during the habituation phase. Panel **(B)** shows that despite a gradual decline in SC responding, CS+/- stimulus discrimination increases across the phase. The first CS+/- trials were removed from analyses of the acquisition phase, as the US had not been encountered, and no learning had occurred. Panels **(C)** and **(D)** show that SCRs to the CS+ and CS- decreased across the early and late extinction phases, respectively.

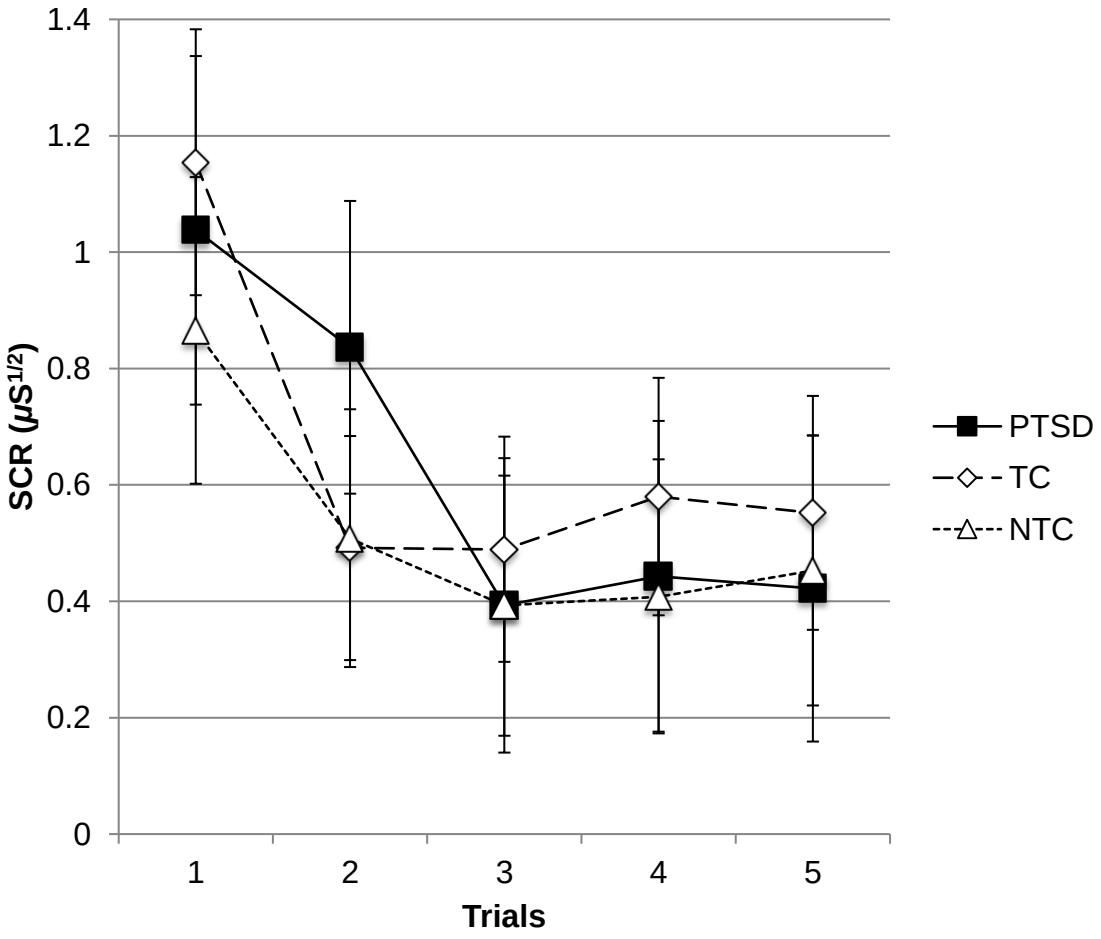

Figure 3. Group × Trial interaction during early extinction. This interaction shows that the PTSD group displayed a slower rate of extinction and safety signal learning across the first 3 to four trials, compared to the TC and NTC groups, who showed a rapid reduction in responding from trial 1 to trial 2. Error bars represent 95% confidence intervals of the mean. Vertical axis displays square-root transformed SCR values ($\mu S^{1/2}$).

5.4.4 Threat expectancy.

Habituation. No significant main effects or interactions were revealed during the habituation phase.

Acquisition. Mixed-model ANOVA revealed a significant CS × trial interaction during the acquisition phase $F(3.40, 254.88) = 103.38$, $p < .001$, $\eta_p^2 = .580$, $\varepsilon = .850$, with differential responding increasing across trials, pooled across groups.

Early extinction. During early extinction, there was also a significant CS × trial interaction, $F(3.46, 262.58) = 10.29$, $p < .001$, $\eta_p^2 = .119$, $\varepsilon = .864$, with differential responding decreasing over early extinction.

Late extinction. During late extinction there was still a significant CS × trial interaction as differential responding continued to decrease, $F(3.63, 275.49) = 3.84$, $p = .006$, $\eta_p^2 = .048$, $\varepsilon = .906$. Furthermore, ANOVA revealed a significant group main effect during late extinction, with the PTSD group reporting higher average US-expectancy ratings than the TC and NTC groups, pooled across CS and trials, $F(2, 76) = 4.76$, $p = .011$, $\eta_p^2 = .111$.

5.4.5 Fear extinction and sAA moderation.

To investigate interactions between fear extinction and sAA in PTSD, we conducted a moderation analysis (Model 1; Hayes, 2013) with the PCL total score as the outcome variable and an early extinction DCR change score served as the predictor variable. sAA levels collected immediately post-acquisition were included in the model as the moderator variable (see Table 2, Model 1). To control for baseline sAA levels, the analysis was repeated with a sAA reactivity score as the moderator (see Table 2, Model 2). As shown in Table 2, the total model did not predict a significant amount of variance in PCL total, $R = 0.12$, $R^2 = .015$, $F(3, 80) = 0.42$, $p = .741$, with no significant main effects of early extinction DCR change or post-acquisition sAA, and no significant moderation interaction. Further, no significant model was found using sAA reactivity as a moderator, $R = 0.12$, $R^2 = .016$, $F(3, 80) = 0.42$, $p = .739$.

Table 2

Linear Model of Predictors of PCL Total.

	b	SE *b*	*t*	*p*
Model 1				
- Constant	29.50 [26.24, 32.77]	1.64	17.98	< .001
- Post-acquisition sAA	-0.32 [-1.23, 0.59]	0.46	-0.70	.485
- Early extinction DCR change	-1.01 [-4.20, 2.17]	1.60	-0.63	.528
- sAA × early extinction	0.24 [-0.62, 1.10]	0.43	0.55	.586
Model 2				
- Constant	29.60 [26.35, 32.86]	1.63	18.12	< .001
- sAA reactivity	-0.47 [-1.69, 0.75]	0.61	-0.77	.445
- Early extinction DCR change	-1.10 [-4.27, 2.08]	1.60	-0.69	.494
- sAA reactivity × early extinction	-0.10 [-1.23, 1.03]	0.57	-0.18	.857

Note: SE = Standard Error; Square brackets show 95% confidence intervals of *b*.

5.5 Discussion

The aim of the current study was to investigate the relationship between endogenous NA activity and fear extinction learning in PTSD, compared to trauma-exposed and non-exposed control groups. First, we found there were no significant between-group differences in baseline salivary NA levels. While there was a significant increase in NA following the acquisition phase and US presentation, this difference did not change as a factor of group. Second, the PTSD group showed significantly slower fear extinction learning during the early extinction phase, compared to the TC and NTC groups. Third, moderation analyses revealed

that NA levels did not moderate the relationship between fear extinction learning and PTSD symptoms.

Increased noradrenergic signaling is known to enhance emotional learning and memory processes (Mueller & Cahill, 2010). Previous research has used behavioral (e.g., Antov et al., 2015; Antov et al., 2013) and pharmacological tasks (e.g., Soeter & Kindt, 2012) designed to activate the stress response and subsequent catecholamine release. The current study, however, measured endogenous NA levels (indexed by sAA) prior to, and immediately following fear acquisition. While the US (in this case, a mild electric shock) appeared to increase NA release, these levels showed no interaction with group or fear extinction learning ability. One possible explanation for this effect is that more sensitive behavioral/pharmacological manipulations may be necessary to increase NA to sufficient levels for measurable effects on fear extinction learning performance. That is, previous research showing relationships between NA and fear conditioning and extinction have either used a specific stress induction task (e.g., cold pressor test; Antov et al., 2015) or pharmacological challenge (e.g., yohimbine and propranolol; Soeter & Kindt, 2012), leading to marked increases or decreases in NA and the sensitivity to observe effects on extinction learning**.**

The findings of the present study show specific PTSD-related impairments in early extinction learning. Specifically, extinction learning appeared to be slower in the PTSD group, compared to TC and NTC groups. These findings support a wealth of evidence of extinction learning deficits in PTSD (Blechert et al., 2007; Norrholm et al., 2011; Orr et al., 2000; Peri et al., 2000; Zuj et al., 2016). Furthermore, we also found a significant group × CS interaction during fear acquisition, showing that the PTSD and TC groups demonstrated elevated fear responding to the CS- compared to the NTC group. This finding indicates that elevated fear expression to a safety signal may be a consequence of trauma exposure, rather

than a PTSD-specific trait. Alternatively, this effect may be the result of enhanced fear generalization as a result of trauma exposure. Previous evidence has suggested that impaired safety signal learning is specific to PTSD (Jovanovic, Kazama, Bachevalier, & Davis, 2012), however the findings of the current study indicate that further research is needed to separate the effects of trauma exposure versus PTSD symptom development.

A limitation of the current study is that we did not include a second day fear extinction recall test, and therefore cannot test the effect that NA release immediately prior to extinction learning had on memory consolidation processes. Previous research used a cold pressor test prior to fear extinction learning to activate the stress response and increase NA release, resulting in a stronger extinction memory trace that was recalled the next day (Antov et al., 2015). Further, evidence has identified a relationship between NA activity and emotional memory consolidation (Nicholson, Bryant, & Felmingham, 2014; Segal & Cahill, 2009). These findings suggest that noradrenergic signaling may be integral in the consolidation of emotionally salient memories (such as conditioned fear memories), rather than the acquisition of fear memories. Future research would benefit from examining the impact of endogenous NA activity in a follow-up extinction recall task (for example, see Shvil et al., 2014). A further limitation is that sAA is an indicator of sympathetic arousal (Bali & Jaggi, 2015; Nater & Rohleder, 2009), and not a direct measure of central NA levels. Although sAA response to physical and psychological stressors corresponds with sympathetic nervous system response patterns (Nater & Rohleder, 2009), a more direct measure of NA activity would strengthen future research in this area. An additional limitation of the current study is the use of participants with subclinical PTSD symptoms. Future research would benefit from a sample consisting exclusively of clinically severe PTSD symptoms for greater sensitivity to find PTSD-specific effects.

In conclusion, the current study found that PTSD is associated with increased fear load, as indicated by significantly slower extinction learning ability during the early trials of fear extinction. Further, while there was an overall increase in NA levels post-fear acquisition, reactive NA levels did not differ as a function of group, and did not interact with fear extinction learning ability. Previous research suggest that behavioral or pharmacological tasks are required for stress-induced NA release that has a significant impact on fear extinction learning, however we cannot conclude on the memory effects of NA release during a standardized differential fear conditioning and extinction paradigm, and we recommend replication of the current methodology with a 2-day fear extinction learning and recall paradigm.

5.6 References

American Psychiatric Association. (2013). *Diagnostic and Statistical Manual of Mental Disorders* (5th ed.). Washington, DC: Author.

Antov, M. I., Melicherová, U., & Stockhorst, U. (2015). Cold pressor test improves fear extinction in healthy men. *Psychoneuroendocrinology, 54*, 54-59. doi:10.1016/j.psyneuen.2015.01.009

Antov, M. I., Wolk, C., & Stockhorst, U. (2013). Differential impact of the first and second wave of a stress response on subsequent fear conditioning in healthy men. *Biological Psychology, 94*(2), 456-468. doi:10.1016/j.biopsycho.2013.08.007

Bali, A., & Jaggi, A. S. (2015). Clinical experimental stress studies: methods and assessment. *Reviews in the Neurosciences, 26*(5), 555-579. doi:10.1515/revneuro-2015-0004

Blechert, J., Michael, T., Vriends, N., Margraf, J., & Wilhelm, F. H. (2007). Fear conditioning in posttraumatic stress disorder: Evidence for delayed extinction of autonomic, experiential, and behavioural responses. *Behaviour Research and Therapy, 45*(9), 2019-2033. doi:10.1016/j.brat.2007.02.012

Cohen, J. (1988). *Statistical power analysis for the behavioural sciences* (2nd ed.). Hillsdale, NJ: Lawrence Erlbaum.

Geracioti, T. D., Jr., Baker, D. G., Ekhator, N. N., West, S. A., Hill, K. K., Bruce, A. B., . . . Kasckow, J. W. (2001). CSF norepinephrine concentrations in posttraumatic stress disorder. *The American Journal of Psychiatry, 158*(8), 1227-1230.

Glover, D. A., & Poland, R. E. (2002). Urinary cortisol and catecholamines in mothers of child cancer survivors with and without PTSD. *Psychoneuroendocrinology, 27*(7), 805-819.

Griffin, M. G., Resick, P. A., & Galovski, T. E. (2012). Does physiologic response to loud tones change following cognitive-behavioral treatment for posttraumatic stress disorder? *Journal of Traumatic Stress, 25*(1), 25-32. doi:10.1002/jts.21667

Hayes, A. F. (2013). *Introduction to mediation, moderation, and conditional process analysis: A regression-based approach*. New York: The Guilford Press.

Highland, K. B., Costanzo, M., Jovanovic, T., Norrholm, S. D., Ndiongue, R., Reinhardt, B., . . . Roy, M. J. (2015). Biomarkers of post-deployment resilience among military service members. *Neurobiology of Stress, 2*, 62-66. doi:10.1016/j.ynstr.2015.07.001

Jovanovic, T., Kazama, A., Bachevalier, J., & Davis, M. (2012). Impaired safety signal learning may be a biomarker of PTSD. *Neuropharmacology, 62*(2), 695-704. doi:10.1016/j.neuropharm.2011.02.023

Lemieux, A. M., & Coe, C. L. (1995). Abuse-related posttraumatic stress disorder: evidence for chronic neuroendocrine activation in women. *Psychosomatic Medicine, 57*(2), 105-115.

Liberzon, I., Abelson, J. L., Flagel, S. B., Raz, J., & Young, E. A. (1999). Neuroendocrine and psychophysiologic responses in PTSD: a symptom provocation study. *Neuropsychopharmacology, 21*(1), 40-50. doi:10.1016/s0893-133x(98)00128-6

Lommen, M. J. J., Engelhard, I. M., Sijbrandij, M., van den Hout, M. A., & Hermans, D. (2013). Pre-trauma individual differences in extinction learning predict posttraumatic

stress. *Behaviour Research and Therapy, 51*(2), 63-67. doi:10.1016/j.brat.2012.11.004

Lovibond, S. H., & Lovibond, P. F. (1995). *Manual for the Depression Anxiety Stress Scales* (2nd ed.). Sydney: Psychology Foundation.

Menz, M. M., Rihm, J. S., Salari, N., Born, J., Kalisch, R., Pape, H. C., . . . Buchel, C. (2013). The role of sleep and sleep deprivation in consolidating fear memories. *Neuroimage, 75*, 87-96. doi:10.1016/j.neuroimage.2013.03.001

Milad, M. R., Orr, S. P., Pitman, R. K., & Rauch, S. L. (2005). Context modulation of memory for fear extinction in humans. *Psychophysiology*, *42*(4), 456-464. doi:10.1111/j.1469-8986.2005.00302.x

Mineka, S., & Oehlberg, K. (2008). The relevance of recent developments in classical conditioning to understanding the etiology and maintenance of anxiety disorders. *Acta Psychologica*, *127*(3), 567-580. doi:10.1016/j.actpsy.2007.11.007

Mueller, D., & Cahill, S. P. (2010). Noradrenergic modulation of extinction learning and exposure therapy. *Behavioural Brain Research, 208*(1), 1-11. doi:10.1016/j.bbr.2009.11.025

Nater, U. M., & Rohleder, N. (2009). Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: Current state of research. *Psychoneuroendocrinology, 34*(4), 486-496. doi:10.1016/j.psyneuen.2009.01.014

National Center for Posttraumatic Stress Disorder. (n.d.). Using the PTSD Checklist for DSM-IV (PCL). Retrieved from http://www.ptsd.va.gov/professional/pages/assessments/assessment-pdf/PCL-handout.pdf

Nicholson, E. L., Bryant, R. A., & Felmingham, K. L. (2014). Interaction of noradrenaline and cortisol predicts negative intrusive memories in posttraumatic stress disorder. *Neurobiology of Learning and Memory, 112*, 204-211. doi:10.1016/j.nlm.2013.11.018

Norrholm, S. D., Jovanovic, T., Olin, I. W., Sands, L. A., Karapanou, I., Bradley, B., & Ressler, K. J. (2011). Fear extinction in traumatized civilians with posttraumatic stress disorder: Relation to symptom severity. *Biological Psychiatry*, *69*(6), 556-563. doi:10.1016/j.biopsych.2010.09.013

Orr, S. P., Metzger, L. J., Lasko, N. B., Macklin, M. L., Peri, T., & Pitman, R. K. (2000). De novo conditioning in trauma-exposed individuals with and without posttraumatic stress disorder. *Journal of Abnormal Psychology, 109*(2), 290-298. doi:10.1037/0021-843x.109.2.290

Peri, T., Ben-Shakhar, G., Orr, S. P., & Shalev, A. Y. (2000). Psychophysiologic assessment of aversive conditioning in posttraumatic stress disorder. *Biological Psychiatry*, *47*(6), 512-519. doi:10.1016/s0006-3223(99)00144-4

Pitman, R. K., Rasmusson, A. M., Koenen, K. C., Shin, L. M., Orr, S. P., Gilbertson, M. W., . . . Liberzon, I. (2012). Biological studies of post-traumatic stress disorder. *Nature, 13*, 769-787. doi:10.1038/nm3339

Rohleder, N., & Nater, U. M. (2009). Determinants of salivary alpha-amylase in humans and methodological considerations. *Psychoneuroendocrinology, 34*(4), 469-485. doi:10.1016/j.psyneuen.2008.12.004

Segal, S. K., & Cahill, L. (2009). Endogenous noradrenergic activation and memory for emotional material in men and women. *Psychoneuroendocrinology, 34*(9), 1263-1271. doi:10.1016/j.psyneuen.2009.04.020

Shvil, E., Sullivan, G. M., Schafer, S., Markowitz, J. C., Campeas, M., Wager, T. D., . . . Neria, Y. (2014). Sex differences in extinction recall in posttraumatic stress disorder: A pilot fMRI study. *Neurobiology of Learning and Memory*. doi:10.1016/j.nlm.2014.02.003

Soeter, M., & Kindt, M. (2012). Stimulation of the noradrenergic system during memory formation impairs extinction learning but not the disruption of reconsolidation. *Neuropsychopharmacology, 37*(5), 1204-1215. doi:10.1038/npp.2011.307

Southwick, S. M., Bremner, J. D., Rasmusson, A., Morgan, C. A., Arnsten, A., & Charney, D. S. (1999). Role of norepinephrine in the pathophysiology and treatment of posttraumatic stress disorder. *Biological Psychiatry, 46*(9), 1192-1204. doi:10.1016/s0006-3223(99)00219-x

Videlock, E. J., Peleg, T., Segman, R., Yehuda, R., Pitman, R. K., & Shalev, A. Y. (2008). Stress hormones and post-traumatic stress disorder in civilian trauma victims: a longitudinal study. Part II: The adrenergic response. *International Journal of Neuropsychopharmacology, 11*(3), 373-380. doi:10.1017/s1461145707008139

Vrana, S. R., & Lauterbach, D. (1994). Prevalence of traumatic events and post-traumatic psychological symptoms in a nonclinical sample of college students. *Journal of Traumatic Stress, 7,* 289-302.

Weathers, F. W., Litz, B. T., Huska, J. A., & Keane, T. M. (1994). *The PTSD Checklist-Civilian Version (PCL-C) for DSM-IV*. Boston: National Center for PTSD, Behavioral Sciences Division.

Yehuda, R., Siever, L. J., Teicher, M. H., Levengood, R. A., Gerber, D. K., Schmeidler, J., & Yang, R. K. (1998). Plasma norepinephrine and 3-methoxy-4-hydroxyphenylglycol concentrations and severity of depression in combat posttraumatic stress disorder and major depressive disorder. *Biological Psychiatry, 44*(1), 56-63.

Young, E. A., & Breslau, N. (2004). Cortisol and catecholamines in posttraumatic stress disorder: an epidemiologic community study. *Archives of General Psychiatry, 61*(4), 394-401. doi:10.1001/archpsyc.61.4.394

Zoladz, P. R., & Diamond, D. M. (2013). Current status on behavioral and biological markers of PTSD: A search for clarity in a conflicting literature. *Neuroscience and Biobehavioral Reviews, 37*(5), 860-895. doi:10.1016/j.neubiorev.2013.03.024

Zuj, D. V., Palmer, M. A., Hsu, C. K., Nicholson, E. L., Cushing, P. J., Gray, K. E., & Felmingham, K. L. (2016). Impaired fear extinction associated with PTSD increases with hours-since-waking. *Depression and Anxiety, 33*(3), 203-210. doi:10.1002/da.22463

Chapter 6

Endogenous Cortisol Reactivity Moderates the Relationship Between Fear Inhibition to Safety Signals and Posttraumatic Stress Disorder Symptoms

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6.1 Abstract

Background: Posttraumatic stress symptoms (PTSS) are commonly associated with impairments in extinguishing fear to signals previously associated with danger, and also with inhibiting fear to safety signals. Previous studies indicate that PTSS are associated with low cortisol activity, and cortisol is shown to facilitate fear extinction. Few studies have examined the influence of cortisol reactivity on fear extinction in PTSS.

Method: We used a standardized fear conditioning and extinction paradigm to investigate the relationship between fear extinction and endogenous salivary cortisol activity in participants with high PTSS (n = 18), trauma-exposed controls (n = 33), and non-trauma-exposed controls (n = 27). Skin conductance response (SCR) was used as an index of conditioned responding. Saliva samples were collected at baseline, and 20 minutes post-fear acquisition for basal and reactive cortisol levels, respectively.

Results: PTSS participants demonstrated a slower rate of extinction learning during the early extinction phase. A moderation analysis revealed that cortisol reactivity was a significant moderator between fear inhibition to the safety signal (CS-) during early extinction and PTSS, but not to the threat signal (CS+). Specifically, this interaction was significant in two ways: (1) participants with elevated cortisol reactivity showed lower PTSS as fear inhibition improved; and (2) participants with low cortisol reactivity showed higher PTSS as fear inhibition improved.

Conclusion: The findings of the present study show that the relationship between fear inhibition and cortisol reactivity is complex, and suggest that cortisol reactivity shapes safety signal learning in PTSS.

6.2 Introduction

Posttraumatic stress disorder (PTSD) develops following a traumatic event that threatens physical integrity (American Psychiatric Association, 2013). Symptoms of PTSD include distressing intrusive memories, avoidance of trauma reminders, negative cognitions and mood, and hyperarousal. A prevailing model posits that persistent PTSD symptoms develop, in part, because of an impaired ability to extinguish a conditioned fear trace (e.g., Mineka & Oehlberg, 2008; Pitman et al., 2012). Stimuli present during the trauma become associated with fear and heightened arousal responses experienced during the event. After the event, benign stimuli act as triggers (conditioned stimuli; CS) for the fear response. PTSD-related impairments in fear extinction learning are well established (Blechert, Michael, Vriends, Margraf, & Wilhelm, 2007; Norrholm et al., 2011; Orr et al., 2000; Peri, Ben-Shakhar, Orr, & Shalev, 2000; Zuj, Palmer, Hsu, et al., 2016), and impaired fear extinction has been proposed as a central construct that links pre-trauma biological and cognitive vulnerability factors to PTSD (Zuj, Palmer, Lommen, & Felmingham, 2016). This is supported by evidence that impaired extinction learning prior to trauma exposure predicts subsequent PTSD following trauma (Guthrie & Bryant, 2006; Lommen, Engelhard, Sijbrandij, van den Hout, & Hermans, 2013; Orr et al., 2012). Further, exposure-based therapies for PTSD act on the principle of extinguishing conditioned fear to trauma-related memory triggers (Milad, Rauch, Pitman, & Quirk, 2006).

Dysregulations in cortisol activity are commonly observed in patients with PTSD (Yehuda, 1997, 2009). The hypothalamic-pituitary-adrenal (HPA) axis is the premier neuroendocrine center involved in the neurochemical release of hormones and neuropeptides to combat stress. The end product of this neurochemical release, cortisol, binds to glucocorticoid receptors (GRs) in a negative feedback loop to inhibit the sympathetic nervous system and ongoing arousal (Yehuda, 1997), thus returning these neural systems to

homeostasis. Alterations in the negative feedback system of the HPA axis have long been hypothesized in PTSD (e.g., Yehuda, Giller, Southwick, Lowy, & Mason, 1991; Yehuda et al., 1993). After controlling for methodological differences (e.g., time-of-day, measurement type), a recent meta-analysis found that PTSD (and PTSD comorbid with major depressive disorder) was associated with lower daily cortisol output relative to non-trauma-exposed controls, and enhanced HPA feedback appears to be associated with general trauma exposure (Morris, Compas, & Garber, 2012). The authors conclude that lower daily cortisol output may be a risk factor for the development of PTSD, rather than an effect of trauma exposure. Nevertheless, an earlier meta-analysis by Miller, Chen, and Zhou (2007) noted that the relationship between cortisol activity and PTSD is complex, and further research in this area is required.

The effect of cortisol release (via pharmacological augmentation or acute stress tasks) on fear extinction learning has received little attention. Studies that have been conducted vary in their methodological design, for example the timing of cortisol administration (or stress induction) during conditioning and extinction paradigms (Merz, Hermann, Stark, & Wolf, 2014). A recent study in healthy participants found that a stress-induction task impaired the retrieval of fear extinction memories, despite no deficit in fear acquisition or extinction 24 hours earlier (Raio, Brignoni-Perez, Goldman, & Phelps, 2014). Studies in rodents have found that glucocorticoid receptor agonists enhance the uptake of cortisol and facilitate fear extinction learning (Yang, Chao, & Lu, 2006). These effects are partly due to actions on glutamate N-methyl-$_{D}$-aspartate (NMDA) receptors in the amygdala (Yang, Chao, Ro, Wo, & Lu, 2007), and many studies have shown glucocorticoid administration facilitates extinction (Barrett & Gonzalez-Lima, 2004; Cai, Blundell, Han, Greene, & Powell, 2006; de Quervain, Aerni, Schelling, & Roozendaal, 2009).

Successful fear extinction is the underlying construct and goal of exposure therapy (Graham & Milad, 2011; Rothbaum & Davis, 2003), and convergent evidence in clinical settings suggest that glucocorticoid administration improves response to exposure treatment for fear-related disorders. For example, clinical patients suffering from spider phobia who were administered hydrocortisone demonstrated significantly reduced fear after undergoing exposure therapy compared with placebo-controls (Soravia et al., 2006; Soravia et al., 2014). Further, Yehuda et al. (2015) have shown that exposure therapy induces current, situational fear during treatment, which in turn increases patient drop out rates and that this can be countered with hydrocortisone administration in conjunction with prolonged exposure therapy. This reduces situational fear expression during the treatment session and thereby reduces drop out rates and enhances treatment benefit (Yehuda et al., 2015). In support, a recent study in patients with panic disorder and agoraphobia demonstrated that elevated endogenous cortisol significantly moderates greater symptom improvement to exposure therapy (Meuret et al., 2015). Together, these findings implicate that elevated cortisol levels facilitate the experimental and therapeutic extinction of fear.

Few studies have examined the influence of cortisol reactivity on fear extinction learning in the context of PTSD. Therefore, the current study used a discrimination fear conditioning and extinction paradigm to investigate the relationship between fear extinction and endogenous salivary cortisol reactivity in a sample with posttraumatic stress disorder symptoms (PTSS) and compared this to trauma-exposed, and non-exposed control groups without PTSS. Based on previous evidence, we considered two ways in which this relationship could manifest. First, greater cortisol reactivity may simply be associated with better fear extinction performance and lower PTSS. The second possibility is that cortisol reactivity may moderate the relationship between fear extinction learning and PTSS. That is, PTSS may be associated with significantly poorer fear extinction learning (e.g., Blechert et

al., 2007; Norrholm et al., 2011), but this relationship may be stronger for participants with lower cortisol reactivity.

6.3 Method

6.3.1 Participants.

Seventy-eight participants aged 18-63 years (*M* = 28.1 years, *SD* = 11.8 years; 35 males and 43 females) comprised three groups: PTSS (*n* = 18), trauma-exposed controls (TC; *n* = 33), and non-trauma-exposed controls (NTC; *n* = 27). Participants were classified on the basis of exposure to a criterion A stressor that threatened physical integrity (American Psychiatric Association, 2013) using the Traumatic Events Questionnaire (TEQ; Vrana & Lauterbach, 1994). Participants were exposed to a variety of environmental and interpersonal traumas: war exposure (*n* = 4), life-threatening accident (*n* = 19), natural disaster (*n* = 24), witness to serious injury or death (*n* = 33), assaulted or molested (*n* = 22), threatened or held captive (*n* = 12), and tortured or terrorist victim (*n* = 1). Mean years since trauma for the PTSS group was 10.5 years (*SD* = 13.7 years), and 9.8 years (*SD* = 11.1 years) for the TC group. The PTSD Checklist-Civilian version (PCL-C; Weathers, Litz, Huska, & Keane, 1994) was used to estimate PTSS severity – participants who presented with at least 1 intrusive memory symptom, 3 avoidance symptoms, and 2 hyperarousal symptoms (defined as a score ≥ 3 on respective PCL-C items; National Center for Posttraumatic Stress Disorder, n.d.) were classified as showing clinical PTSS according to diagnostic criteria of the DSM-IV. Following these criteria, all PTSS participants had a PCL-C total greater than 40, and 61% (*n* = 11) of the PTSS group showed a PCL-C total greater than 50. The PCL-C for DSM-IV was used as testing began prior to the release of diagnostic instruments for the DSM-5. Participants were regarded as TC if they reported experience of a criterion A stressor, but did not reach the minimum possible cutoff of 30 for PTSD in general population

samples (National Center for Posttraumatic Stress Disorder, n.d.). All participants in the TC group showed a PCL-C total score ≤ 29. Participants who reported no lifetime experience of a criterion A stressor were classified as NTC. Participants also completed the Depression Anxiety Stress Scale-21 item version (DASS; Lovibond & Lovibond, 1995). The Tasmanian Health and Medical Research Ethics Committee and the University of Tasmania Social Science Human Research Ethics Committee approved this study, and all participants gave full informed consent.

6.3.2 Fear conditioning and extinction paradigm.

The present study employed a differential fear conditioning and extinction paradigm used previously (Orr et al., 2000; Zuj, Palmer, Hsu, et al., 2016). Findings from a subset of the participants in the current study have been published elsewhere, which examined the impact of time-since-waking on fear extinction learning (Zuj, Palmer, Hsu, et al., 2016). The unconditioned stimulus (US) was a 500ms mild electric shock delivered to the first interosseous muscle of the dominant hand, and set to a level considered "highly annoying, but not painful" by each participant prior to the task (Orr et al., 2000; Zuj, Palmer, Hsu, et al., 2016). Conditioned stimuli (CS) were red and blue circles presented individually for 12s on a computer screen. The testing protocol included four experimental phases: *habituation*, *acquisition*, *early extinction*, and *late extinction*. During *habituation*, participants were exposed to four trials of each colored circle (eight trials in total). After a short pause, participants were prompted to begin the *acquisition* phase when ready. During this phase, one of the colored circles (CS+) was followed by the US on all five trials (100% reinforcement schedule) while the other colored circle was not reinforced on any of the five trials (CS-; ten trials in total). There was a short interval of approximately 2-3 minutes post-acquisition, before participants completed the *early* and *late extinction* phases. The *early extinction* phase

consisted of five trials of the CS+ (with no reinforcement) and five trials of the CS- (ten trials in total), followed by the *late extinction* phase, which mirrored early extinction. There was an approximately 1 minute rest period between early and late extinction (Milad, Orr, Pitman, & Rauch, 2005). Trial order was pseudo-random, with no more than two consecutive CS+ or CS- trials, and a variable inter-trial interval was used, ranging from 12-21 seconds (M = 16 seconds). All phases were completed in a single testing session. To ensure contingency awareness, participants were asked at the end of testing which colored circle was associated with the US. All participants reported accurate contingency awareness.

6.3.3 Skin conductance.

Skin conductance level (SCL) was measured through a 22mV_{rms}, 75Hz constant-voltage coupler (FE116, ADInstruments) with bipolar electrodes on the intermediate phalange of the first and third fingers of the non-dominant hand, sampled at 512Hz and stored at 64Hz, and recorded in micro-siemens (μS). Skin conductance response (SCR) to the CS+ and CS- was calculated by subtracting the mean SCL during the 2s prior to stimulus onset from the maximum SCL during the 12s stimulus duration. SCR values were square-root transformed to normalize distributions (for negative SCR values, the absolute value was square-root transformed and the negative sign replaced).

6.3.4 US-expectancy ratings.

During each 12s stimulus presentation, participants were asked to rate their expectancy of the US on a 0-100 visual analogue scale (0 "certain no electrical stimulus"; 100 "certain electrical stimulus"; as used previously by Lommen et al., 2013).

6.3.5 Salivary cortisol.

Saliva for cortisol was collected twice during testing. The first sample was collected upon arrival to the lab, and the second sample was collected approximately 20 minutes after the fear acquisition phase. Samples were immediately transferred to a freezer. Prior to assay, the saliva was thawed and centrifuged, and cortisol was measured using a commercially available ELISA assay (Salimetrics, USA) according to the manufacturers instructions. Salivary cortisol showed an assay sensitivity of 0.003 μg/dL. Intra-assay variability was 4.1%, and inter-assay variability was 4.6%. Cortisol data were square-root transformed to normalize distributions. A cortisol reactivity score was calculated by subtracting baseline levels from post-acquisition levels. To control for circadian variation in cortisol, testing was conducted between 12-6PM. Time-of-testing and hours-since-waking did not differ significantly between groups (p = .114, and p = .610, respectively).

6.3.6 Statistical analyses.

Separate 3 (group) × 2 (CS) × 5 (trial) mixed-model analyses of variance (ANOVA) were conducted for each phase (with four trials for habituation and acquisition) to examine fear conditioning and extinction across groups. The first CS+/- trials during the acquisition phase were removed from analyses as the US had not been encountered at this stage, and no fear learning would have occurred (Zuj, Palmer, Hsu, et al., 2016) Analyses were identical for both the SCR and US-expectancy data. Greenhouse-Geisser corrections were made for within-subjects variables where necessary. Brown-Forsythe F-ratio adjustments were made where necessary, and pairwise comparisons were conducted with Bonferroni corrections or Games-Howell tests where appropriate. Moderation analyses were conducted using the PROCESS macro for SPSS (Model 1; Hayes, 2013). An alpha level of α = .05 was used for all tests of statistical significance. Effect sizes are reported as Cohen's d following the criteria

of 0.2, 0.5, and 0.8 as small, moderate, and large effects, respectively (Cohen, 1988). Partial-eta squared (η_p^2) are reported as effect sizes for mixed-model ANOVAs.

6.4 Results

6.4.1 Descriptive and clinical data.

Descriptive statistics, and additional inferential data are displayed in Table 1. One-way ANOVA revealed a significant between-group difference on age, $F(2, 42.51) = 4.15$, $p = .023$. While there was no significant age difference between the PTSS and TC groups ($p = .16$), and between the TC and NTC groups ($p = .47$), the PTSS group was, on average, significantly older than NTC participants ($p = .037$). As expected, there were significant between-group differences in PCL total scores, $F(2, 21.17) = 122.98$, $p < .001$. The PTSS group showed a significantly higher mean PCL total than the TC and NTC groups ($ps < .001$), who also significantly differed ($p < .001$). One-way ANOVA revealed significant between-group differences on the depression, anxiety and stress subscales of the DASS (see Table 1 for descriptive and inferential statistics). Games-Howell post-hoc tests revealed that the PTSS group displayed significantly higher levels of depression, anxiety and stress than the TC and NTC groups (all $ps < .001$). The TC group also had significantly higher levels of stress than the NTC group ($p = .003$), with no significant differences on depression or anxiety between these two groups.

Table 1

Mean Scores and SDs for Demographic and Clinical Variables.

Measures	PTSD (*n* = 18)	TC (*n* = 33)	NTC (*n* = 27)	Test statistic	*P*-value
Demographic data					
- Age (years)	34.9 (15.0)	27.5 (10.1)	24.4 (9.7)	$F(2, 42.51) = 4.15$	.023
- Sex	11F, 7M	14F, 19M	18F, 9M	$\chi^2(2) = 3.87$	.145
PCL-C					
- Total	54.33 (11.81)	23.45 (3.85)	19.96 (2.52)	$F(2, 21.17) = 122.98$	< .001
- Intrusive	3.38 (1.26)	0.19 (0.40)	0.00 (0.00)	$F(2, 58) = 132.30$	< .001
- Avoidance	4.15 (1.82)	0.39 (0.67)	0.29 (0.59)	$F(2, 16.55) = 48.12$	< .001
- Hyperarousal	3.77 (1.17)	0.48 (0.81)	0.18 (0.53)	$F(2, 25.09) = 74.56$	< .001
DASS					
- Depression	9.78 (6.16)	2.27 (2.35)	1.63 (2.15)	$F(2, 24.67) = 23.94$	< .001
- Anxiety	8.28 (4.10)	1.88 (1.85)	1.19 (1.66)	$F(2, 27.72) = 37.75$	< .001
- Stress	13.11 (5.62)	5.00 (3.08)	2.59 (2.37)	$F(2, 30.92) = 37.51$	< .001
AUDIT	7.06 (5.61)	6.27 (3.80)	6.22 (4.32)	$F(2, 45.78) = 0.21$	.813

Note: PCL-C, PTSD Checklist; DASS, Depression Anxiety Stress Scale; AUDIT, Alcohol Use Disorders Identification Test.

6.4.2 Salivary cortisol.

Three (group) × 2 (time) repeated measures ANOVA revealed no significant change in cortisol levels from baseline to post-acquisition, $F(1, 73) = 0.06$, $p = .81$. Further, there was no significant group × time interaction, $F(2, 73) = 0.10$, $p = .91$. Raw baseline and post-acquisition cortisol levels are displayed in Figure 1.

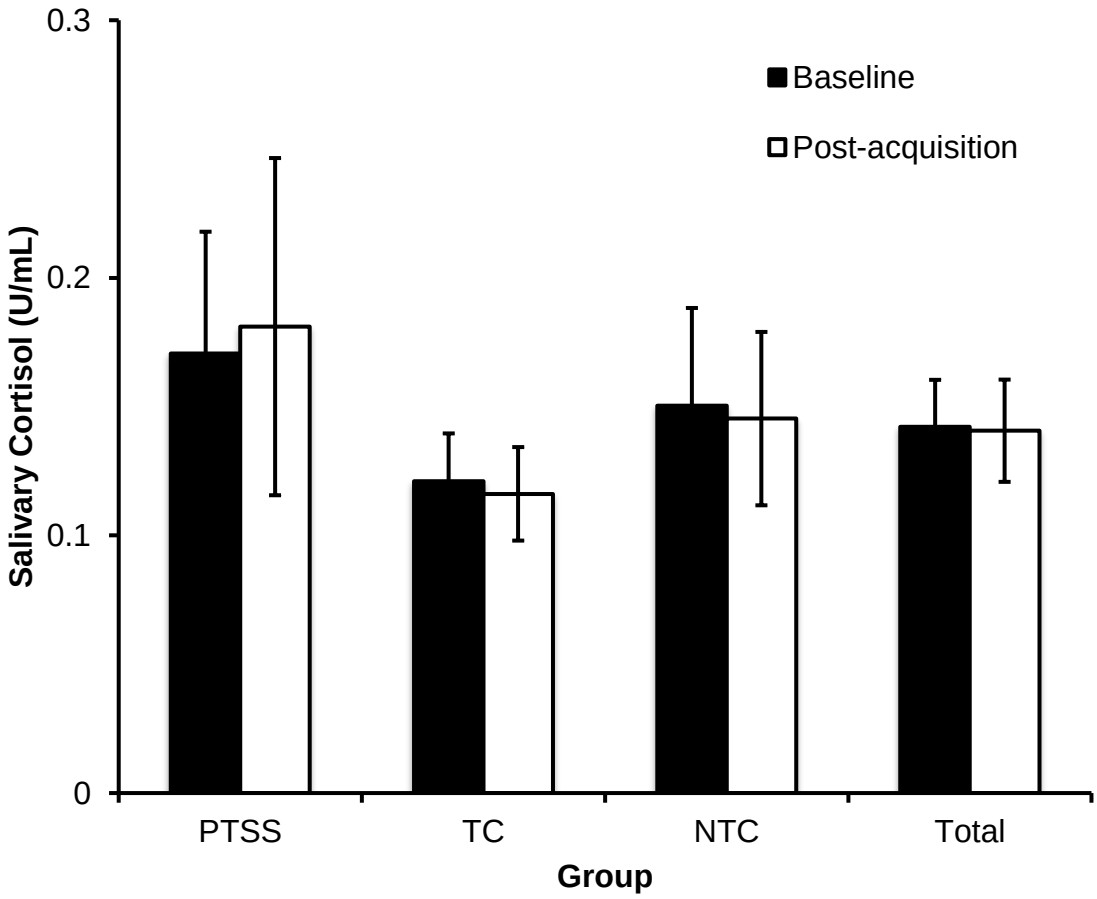

Figure 1. Group and total levels of baseline and post-acquisition cortisol. On average, groups did not significantly differ, and there were no significant changes from baseline to post-acquisition. Figure shows raw salivary cortisol values. Error bars represent 95% confidence intervals.

6.4.3 SCR amplitude data.

Habituation. Mixed-model ANOVA showed a trend-level main effect of trial during the habituation phase, $F(2.85, 213.85) = 2.52$, $p = .06$, $\eta_p^2 = .032$, with SCR levels reducing across all trials. All further main effects and interactions were nonsignificant (*p*s > .05).

Acquisition. Mixed-model ANOVA showed a significant main effect of CS, $F(1, 75) = 67.01$, $p < .001$, $d = 0.83$, with significantly greater responding to the CS+ ($M = 0.84$, 95% CI[0.70, 0.97], $SD = 0.60$) compared to the CS- ($M = 0.38$ [0.27, 0.50], $SD = 0.49$) suggesting the acquisition of a conditioned fear response. There was also a significant main effect of trial, with SCRs declining across the acquisition phase, $F(2.85, 213.55) = 3.55$, $p = .017$, $\eta_p^2 = .045$. There were no further significant interactions, and no significant *F*-tests involving group.

Early extinction. During early extinction there was a significant main effect of CS, $F(1, 75) = 6.47$, $p = .013$, $d = 0.24$, indicating that participants continued displaying significantly greater SCRs in early extinction to the CS+ ($M = 0.59$ [0.50, 0.69], $SD = 0.42$) compared to the CS- ($M = 0.49$ [0.39, 0.59], $SD = 0.43$). Figure 2 shows that, on average, there was a reduction in SCR to the CS+/- from trial 1 to trial 5, as reflected in a significant main effect of trial, $F(3.58, 268.14) = 27.76$, $p < .001$, $\eta_p^2 = .270$. Further, there was a significant group × trial interaction, $F(7.15, 268.14) = 2.63$, $p = .012$, $\eta_p^2 = .065$. Tests of simple main effects revealed that the TC and NTC groups showed a significant reduction in SCRs from trial 1 to trial 2 (*p*s < .001), with no significant change in responding thereafter. The PTSS group, however, displayed a trend-level reduction in SCRs from trial 1 to trial 2 ($p = .051$), and a significant reduction from trial 2 to trial 3 ($p = .006$), suggestive of a slower rate of extinction learning that continued to decline from trial 2 to trial 3.

Late extinction. Mixed-model ANOVA revealed that the CS main effect was no longer significant, $F(1, 75) = 2.79$, $p = .10$, $d = 0.15$. A significant main effect of trial remained, $F(3.26, 244.66) = 13.93$, $p < .001$, $\eta_p^2 = .157$, where responding to both the CS+ and CS- declined across the five trials (see Figure 2). No further main effects or interactions were significant.

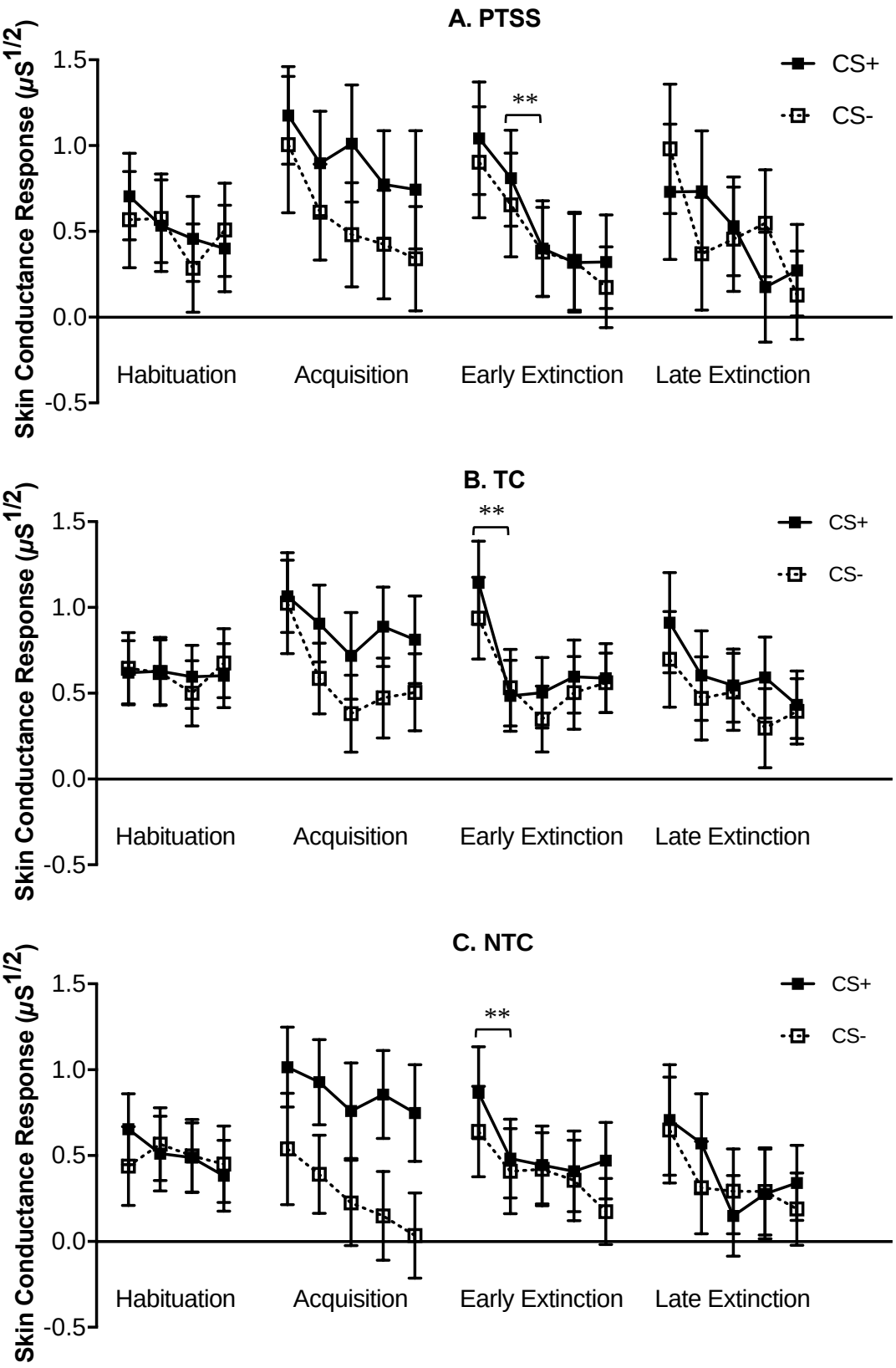

Figure 2. Panel **(A)** displays SCRs to the CS+ and CS- during the fear conditioning and extinction paradigm for the PTSS group. Panel **(B)** displays SCRs for the TC group, and

panel **(C)** displays SCRs for the NTC group. The first CS+/- trial of acquisition was omitted from all statistical analyses as the US had not yet been encountered, and no fear learning had occurred. The first trial of acquisition has been included in the figure for general information on the fear learning processes between PTSD, TC, and NTC groups. The NTC group displayed greater SCR to the CS+ compared to the CS- on trial 1 of acquisition, which was not anticipated. There was a significant group × trial interaction during the early extinction phase, with participants in the PTSD group showing a trend-level reduction from trial 1 to trial 2, and a significant reduction from trial 2 to trial 3, compared to the TC and NTC groups, who showed a significant reduction of SCRs from trial 1 to trial 2. Error bars display 95% confidence intervals of the mean. $\mu S^{1/2}$, SCR square-root transformed in micro-siemens. ** $p < .01$.

6.4.4 Threat expectancy ratings.

Habituation. A 3 × 2 × 4 mixed-model ANOVA revealed a significant main effect of trial during habituation, $F(2, 139.14) = 5.38$, $p = .006$, $\eta_p^2 = .071$, with US-expectancy ratings gradually declining as participants habituated to the task.

***Acquisition*.** During acquisition, there were significant main effects of CS, $F(1, 69) = 235.42$, $p < .001$, $d = 3.04$, and trial, $F(3.50, 241.23) = 3.29$, $p = .016$, $\eta_p^2 = .046$, $\varepsilon = .874$. The successful acquisition of fear was evidenced by a significant CS × trial interaction, $F(3.50, 241.66) = 82.67$, $p < .001$, $\eta_p^2 = .545$, with differential responding to the CS+/- increasing throughout acquisition. That is, threat expectancy to the CS+ increased while responding to the CS- decreased, as shown in Figure 3.

Early extinction. During early extinction, ANOVA revealed significant main effects of CS, $F(1, 70) = 46.96$, $p < .001$, $d = 0.97$, and trial, $F(2.53, 176.87) = 65.27$, $p < .001$, $\eta_p^2 = .483$, $\varepsilon = .632$. These main effects were superseded by a significant CS × trial interaction, $F(3.39, 236.96) = 7.16$, $p < .001$, $\eta_p^2 = .093$, $\varepsilon = .846$, with differential responding reducing across the early extinction phase (see Figure 3).

Late extinction. Similar to early extinction, the late extinction phase showed a significant main effect of CS, $F(1, 70) = 18.10$, $p < .001$, $d = 0.47$. There was a significant trial main effect, $F(2.18, 152.72) = 39.40$, $p < .001$, $\eta_p^2 = .360$, $\varepsilon = .545$. ANOVA also revealed a significant CS × trial interaction, $F(3.43, 239.95) = 3.95$, $p = .006$, $\eta_p^2 = .053$, showing that the differential threat expectancy declined from trial 1 to trial 5 during the late extinction phase. Further, during late extinction there was a significant group main effect, $F(2, 70) = 8.98$, $p < .001$, $\eta_p^2 = .204$, with the PTSS group displaying higher mean US-expectancy ($M = 47.24$ [37.40, 57.07], $SD = 20.33$) than TC and NTC groups ($M = 23.07$ [15.78, 30.35], $SD = 20.33$, and $M = 23.80$ [15.69, 31.91], $SD = 20.33$, respectively).

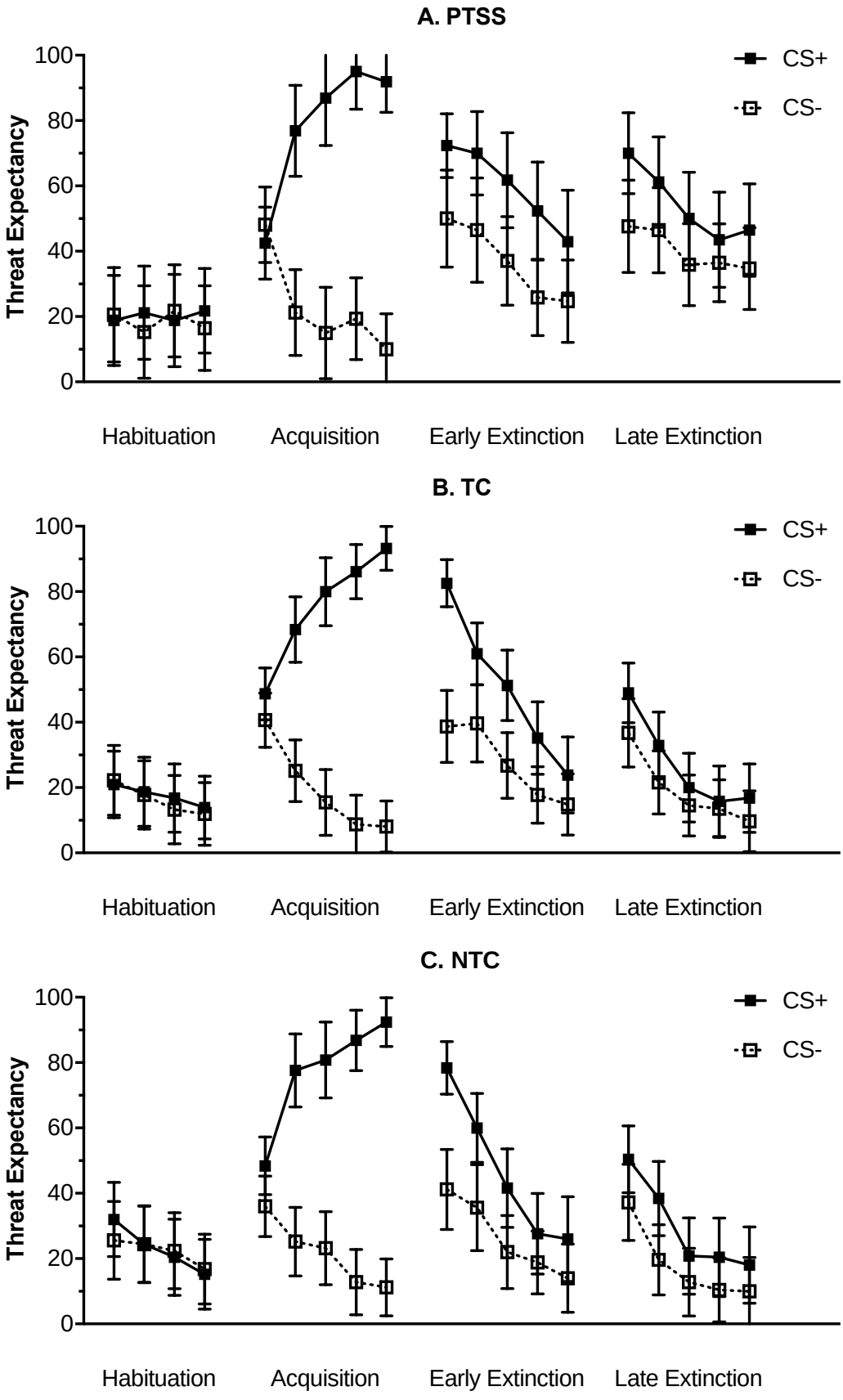
A. PTSS
B. TC
C. NTC
Threat Expectancy
100
80
60
40
20
0
CS+
CS-
Habituation
Acquisition
Early Extinction
Late Extinction

Figure 3. Threat expectancy ratings for the CS+ and CS- across the differential conditioning and extinction paradigm are displayed separately for the PTSS group **(A)**, TC group **(B)**, and NTC group **(C)**. The acquisition panels show clear understanding of the CS+/US contingency, with scores greater than 50 representing increased threat expectancy, and scores lower than 50 representing reduced threat expectancy. The early and late extinction panels show that threat expectancy of the US attenuated for all three groups. Error bars display 95% confidence intervals of the mean.

6.4.5 Moderation analyses.

As a significant group × trial interaction effect on SCR was only found during early extinction, and the three groups were all displaying similar levels of extinction learning by trial 3, the difference between trial 1 and trial 2 was calculated separately for the CS+ and the CS-, and entered into separate moderation analyses as the predictor variables (see Table 2, Models 1 and 2, respectively). Cortisol reactivity was entered as the moderator, with PCL total as the outcome variable, and age and depression were included as covariates. Using the predictor derived from the CS+ change score, there was a significant total model, $R^2 = .660$, $F(5, 70) = 27.18$, $p < .001$. However, as seen in Table 2, model 1, there was no significant effect of cortisol reactivity or CS+ change, and there was no significant moderation interaction between these variables. The main effects and moderation interaction remained nonsignificant after including age and depression as covariates.

Table 2

Linear Model of Predictors of PCL Total.

	b	SE *b*	*t*	*p*
Model 1				
- Constant	11.63 [6.02, 17.25]	2.82	4.13	< .001
- Cortisol reactivity	3.99 [-20.48, 28.45]	12.27	0.33	.746
- CS+ change T1-T2	-1.94 [-5.12, 1.24]	1.60	-1.21	.229
- CS+ change × Cortisol reactivity	6.34 [-33.19, 45.88]	19.82	0.75	.750
Model 2				
- Constant	11.65 [6.32, 16.99]	2.67	4.36	< .001
- Cortisol reactivity	-0.81 [-23.79, 22.16]	11.52	-0.07	.944
- CS- change T1-T2	0.53 [-2.06, 3.12]	1.30	0.41	.685
- CS- change × Cortisol reactivity	-46.44 [-78.17, -14.71]	15.91	-2.92	.005

Note: SE = standard error; Square brackets show 95% confidence intervals of *b*; * interaction is significant after age and depression were included in the model as covariates.

Using the early extinction CS- change from trial 1 to trial 2 as the predictor, the model predicted a significant amount of variance in PCL total, $R^2 = .690$, $F(5, 70) = 31.08$, $p < .001$. There were no significant main effects of cortisol reactivity or CS- change. Critically, however, cortisol reactivity was a significant moderator of the relationship between CS- change and PCL total (see Table 2, model 2), with age and depression included as covariates. As shown in Figure 4, participants with greater cortisol reactivity showed a negative relationship between fear extinction learning and PTSS. That is, for participants with lower PTSS severity, high cortisol reactivity was associated with facilitated fear extinction learning to the CS-. This relationship was not evident at lower levels of cortisol reactivity: For participants with moderate levels of cortisol reactivity, fear extinction learning was not significantly related to PTSD symptoms. And, interestingly, for participants with low cortisol

reactivity, the inverse relationship was found, with higher PTSD symptoms associated with better fear extinction.

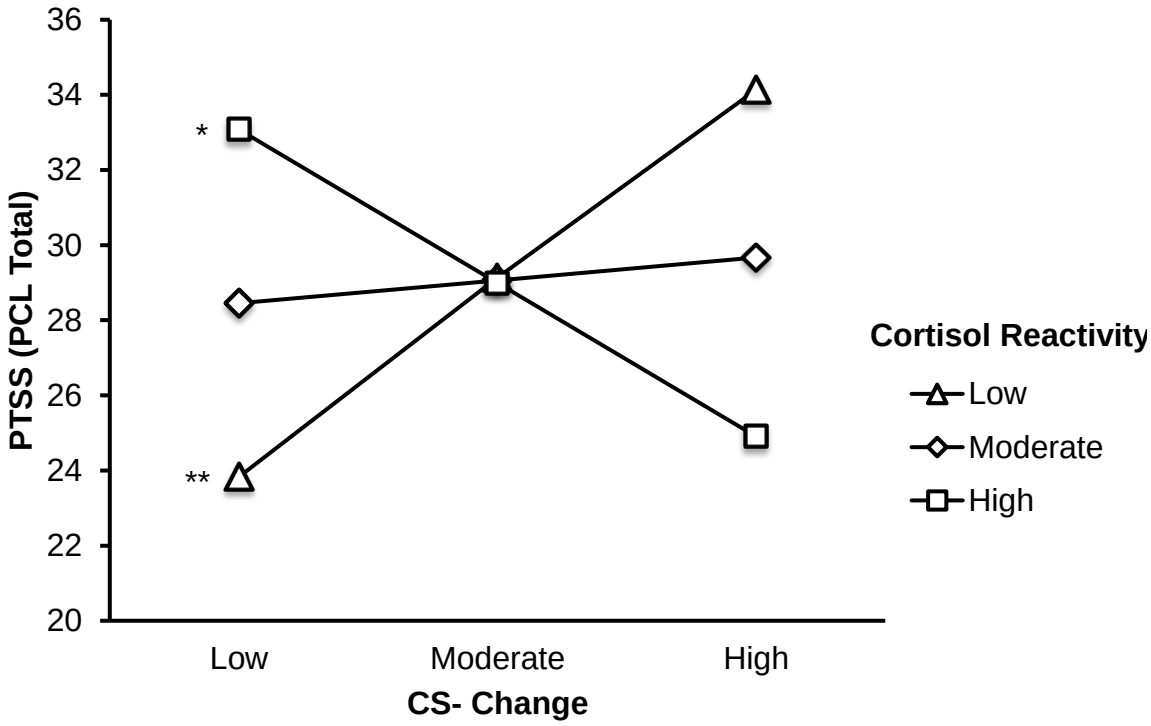

Figure 4. The relationship between early extinction CS- change and PTSS was significant at lower levels (-1.5 *SD*) and higher levels (+1.5 *SD*) of cortisol reactivity. At moderate (mean) levels of cortisol reactivity, there was no significant relationship between fear extinction learning and PTSS. These findings suggest a positive relationship between PTSS and extinction learning when cortisol reactivity is low. Further, there is a negative relationship between PTSS and extinction learning when cortisol reactivity is high. * $p < .05$. ** $p < .01$.

6.5 Discussion

The aim of the current study was to investigate the relationship between fear extinction learning and endogenous cortisol reactivity in PTSS. We found that cortisol reactivity was a significant moderator of the relationship between fear extinction (specifically to the safety signal) and PTSS. This effect was demonstrated in two ways. (1) High cortisol reactivity was associated with lower PTSS severity as fear extinction learning to the safety signal improved; and (2) low cortisol reactivity was associated with higher PTSS severity as fear extinction learning to the safety signal improved. Interestingly, this effect was only found with fear responding to the CS-, rather than the CS+.

In the present study, cortisol reactivity was a significant moderator between fear extinction to the CS- (but not the CS+) and PTSS. During the extinction phase(s) of a simple discrimination paradigm as employed in the current study, appropriate responding to the CS+ and CS- reflect extinction learning and fear inhibition, respectively (Jovanovic & Norrholm, 2011). Therefore, we speculate that the moderation analysis of the current study revealed cortisol reactivity to specifically interact with processes of fear inhibition in PTSS severity. Recent research implicates impaired fear inhibition to safety signals to be a key biomarker in PTSD (Jovanovic, Kazama, Bachevalier, & Davis, 2012; Jovanovic & Norrholm, 2011). Previous research has shown patients with PTSD tend to show generalized conditioned fear responding from a reinforced CS+ to a non-reinforced CS- (Grillon & Morgan, 1999). This effect has previously been hypothesized to be attributable to either difficulty in learning the CS/US contingency, or an error in appropriately inhibiting fear despite accurate contingency awareness (Jovanovic et al., 2009). The findings of the present study support the latter, as all participants reported awareness of the CS/US contingency, a fact that is further supported by the self-reported threat expectancy ratings during the acquisition and early extinction phases (see Figure 3). Further, we previously demonstrated that PTSD is associated with impaired

fear extinction learning, which becomes worse with greater hours-since-waking (Zuj, Palmer, Hsu, et al., 2016), and increased endogenous cortisol activity has recently been shown to mediate better treatment response in the morning in a sample of patients with panic disorder and agoraphobia (Meuret et al., 2016). In the current study, however, participants in the PTSS group showed a general impairment in fear responding to both CS+/- during early extinction, with no specific extinction deficit to either CS. Rather, the findings of the current study showed that cortisol reactivity interacts with fear inhibition to the CS- (but not extinction to the CS+) in a model of PTSS severity.

Regarding the influence of cortisol, the findings of the current study are supported by recent work showing that endogenous cortisol levels significantly moderate (Meuret et al., 2015) and mediate (Meuret et al., 2016) exposure-therapy success in patients with panic disorder and agoraphobia. Pharmacological studies have also demonstrated that dexamethasone suppression of HPA axis function significantly reduces exaggerated fear-potentiated startle in patients with PTSD, compared to traumatized controls without PTSD (Jovanovic et al., 2011). Interestingly, this finding was only revealed for fear-potentiated startle to the CS+ and not the CS-, suggesting a role for endogenous cortisol reactivity in fear inhibition. The findings of the current study, that endogenous cortisol reactivity is a moderator of fear inhibition, but not fear extinction learning, is a novel result, and requires further investigation. Additionally, glucocorticoid administration (significantly increasing cortisol output) in conjunction with exposure therapy for specific phobia significantly reduced symptom severity compared to the placebo control group in keeping with earlier research (de Quervain et al., 2009). Together, these findings suggest that cortisol augmentation and enhancing safety signal learning during the therapy process may improve treatment response. Although salivary cortisol has recently been used in similar research

(Meuret et al., 2016; Meuret et al., 2015), the collection of 24h blood samples would provide a more accurate indication of the role of cortisol in fear extinction or inhibition.

The standardized differential fear conditioning and extinction paradigm used in the current study produced reliable fear conditioning and extinction learning. These findings are in line with previous studies showing PTSD-related impairments in extinction learning (Blechert et al., 2007; Norrholm et al., 2011; Orr et al., 2000; Peri et al., 2000), although some studies have failed to find specific extinction learning deficits in PTSD (Milad et al., 2008; Milad et al., 2009). The findings of the present study are consistent with the idea of "fear load", that PTSD is associated with significantly increased fear expression during the early trials of extinction learning (Norrholm et al., 2015; Norrholm et al., 2011). However, extinction learning in the current study occurred rapidly, with the PTSS group extinguishing conditioned fear responses by trial 3 of early extinction, and by trial 2 for the control groups with relatively small effect sizes. We speculate that this rapid extinction learning may be due to the use of a 100% reinforcement schedule during fear acquisition. While full reinforcement schedules are commonly used (e.g., Jovanovic et al., 2014; Orr et al., 2012; Orr et al., 2000), replicating the current study using less predictable US-exposure (e.g., 60% reinforcement schedule, as used by Milad et al., 2007; Pace-Schott et al., 2013) may slow extinction learning and increase the sensitivity to reveal effects.

The current finding that better fear extinction performance is associated with lower PTSS in the context of higher cortisol reactivity is consistent with the findings of a study that found glucocorticoid administration enhanced exposure therapy outcome in specific phobia (de Quervain et al., 2011). Exposure therapy is one of the most recommended treatments for PTSD (Foa, Keane, Friedman, & Cohen, 2009) and acts by extinguishing conditioned fear memories associated with the trauma (Graham & Milad, 2011; Yehuda et al., 2015). Recently, Yehuda et al. (2015) found that hydrocortisone augmentation of exposure therapy

resulted in significantly greater PTSD symptom reduction and patient retention compared to placebo. Due to increased PTSD symptom expression during initial exposure therapy sessions, patient drop-out rates are a serious issue (e.g., Jeffreys et al., 2014; Yehuda et al., 2015), and the results of the current study suggest that hydrocortisone augmentation of exposure therapy may have been effective in part due to the interaction between altered cortisol levels and correcting impaired fear inhibition processes.

While the primary finding of the moderation analysis is in line with previous research (e.g., Meuret et al., 2015), the moderation also revealed that for participants with low cortisol reactivity, greater PTSS was associated with better fear inhibition. This effect was not expected, and we speculate that low cortisol reactivity may act as a moderator of poor response to exposure therapy in clinical settings. That is, approximately 15-45% of patients still show diagnostic criteria for PTSD after prolonged exposure therapy (e.g., Mørkved et al., 2014; Powers, Halpern, Ferenschak, Gillihan, & Foa, 2010). We suggest that prolonged exposure therapy may target key fear-related symptoms of PTSD, but may be ineffective in the treatment of other symptoms when HPA function is hypoactive, leading to ongoing need for treatment. Glucocorticoid administration may be beneficial in such situations. Alternatively, previous research in the glucocorticoid receptors of the rat nucleus of the solitary tract revealed a dose-response relationship with memory consolidation, whereby intermediate doses enhanced consolidation with no effects at low or high doses (Roozendaal, Williams, & McGaugh, 1999). The moderation effect of low cortisol reactivity on the relationship between fear inhibition and PTSD symptoms was not anticipated, and preliminary animal evidence suggest that glucocorticoid receptors, and possibly cortisol activity, follow a U-curve dose-response relationship with learning and memory processes.

These effects notwithstanding, some limitations were present in the current study. First, we only examined the relationship between cortisol reactivity and fear extinction

learning, and a previous pilot study suggests that a one-month course of low-dose cortisol administration in patients with PTSD resulted in reduced traumatic memory symptoms compared to placebo (de Quervain, 2008). Future research using a multi-day extinction learning and recall paradigm (e.g., Milad et al., 2008; Milad et al., 2009) may reveal important insights into this issue. Second, the PTSS group used in the current study also included participants with subclinical symptoms, and a sample of greater clinical severity are necessary to make robust implications for the interaction between cortisol and fear extinction in a clinical treatment setting. Finally, the use of self-report diagnostic instruments (and diagnostic tools for the DSM-IV) is a limitation of the current study, and future research would benefit from clinical diagnostic tools, such as the Clinician Administered PTSD Scale (CAPS) for the DSM-5.

In conclusion, we observed that endogenous cortisol reactivity is a significant moderator between the inhibition of fear responses to the CS- (but not the CS+) and PTSS. Our findings provide evidence that elevated cortisol influences the relationship between PTSS and fear inhibition, such that greater fear inhibition to a safety signal is associated with lower PTSS, and this relationship only occurs in participants experiencing greater cortisol reactivity. This finding provides a novel insight into the processes of fear inhibition, suggesting that endogenous cortisol reactivity moderates the relationship between fear inhibition to safety signals and PTSS.

6.6 References

American Psychiatric Association. (2013). *Diagnostic and Statistical Manual of Mental Disorders* (5th ed.). Washington, DC: Author.

Barrett, D., & Gonzalez-Lima, F. (2004). Behavioral effects of metyrapone on Pavlovian extinction. *Neurosci Lett, 371*(2-3), 91-96. doi:10.1016/j.neulet.2004.08.046

Blechert, J., Michael, T., Vriends, N., Margraf, J., & Wilhelm, F. H. (2007). Fear conditioning in posttraumatic stress disorder: Evidence for delayed extinction of autonomic, experiential, and behavioural responses. *Behaviour Research and Therapy, 45*(9), 2019-2033. doi:10.1016/j.brat.2007.02.012

Cai, W. H., Blundell, J., Han, J., Greene, R. W., & Powell, C. M. (2006). Postreactivation glucocorticoids impair recall of established fear memory. *Journal of Neuroscience, 26*(37), 9560-9566. doi:10.1523/jneurosci.2397-06.2006

Cohen, J. (1988). *Statistical power analysis for the behavioural sciences* (2nd ed.). Hillsdale, NJ: Lawrence Erlbaum.

de Quervain, D. J. (2008). Glucocorticoid-induced reduction of traumatic memories: implications for the treatment of PTSD. *Progressive Brain Research, 167*, 239-247. doi:10.1016/s0079-6123(07)67017-4

de Quervain, D. J., Aerni, A., Schelling, G., & Roozendaal, B. (2009). Glucocorticoids and the regulation of memory in health and disease. *Frontiers in Neuroendocrinology, 30*(3), 358-370. doi:10.1016/j.yfrne.2009.03.002

de Quervain, D. J., Bentz, D., Michael, T., Bolt, O. C., Wiederhold, B. K., Margraf, J., & Wilhelm, F. H. (2011). Glucocorticoids enhance extinction-based psychotherapy. *Proceedings of the National Academy of Science of the United States of America, 108*(16), 6621-6625. doi:10.1073/pnas.1018214108

Foa, E. B., Keane, T. M., Friedman, M. J., & Cohen, J. A. (Eds.). (2009). *Effective treatments for PTSD: Practice guidelines from the International Society of Traumatic Stress Studies* (2nd ed.). New York: Guilford.

Graham, B. M., & Milad, M. R. (2011). The study of fear extinction: Implications for anxiety disorders. *The American Journal of Psychiatry, 168*(12), 1255-1265. doi:10.1176/appi.ajp.2011.11040557

Grillon, C., & Morgan, C. A., 3rd. (1999). Fear-potentiated startle conditioning to explicit and contextual cues in Gulf War veterans with posttraumatic stress disorder. *Journal of Abnormal Psychology, 108*(1), 134-142.

Guthrie, R. M., & Bryant, R. A. (2006). Extinction learning before trauma and subsequent posttraumatic stress. *Psychosomatic Medicine, 68*, 307-311. doi:10.1097/01.psy.0000208629.67653.cc

Hayes, A. F. (2013). *Introduction to mediation, moderation, and conditional process analysis: A regression-based approach*. New York: The Guilford Press.

Jeffreys, M. D., Reinfeld, C., Nair, P. V., Garcia, H. A., Mata-Galan, E., & Rentz, T. O. (2014). Evaluating treatment of posttraumatic stress disorder with cognitive processing therapy and prolonged exposure therapy in a VHA specialty clinic. *Journal of Anxiety Disorders, 28*(1), 108-114. doi:10.1016/j.janxdis.2013.04.010

Jovanovic, T., Kazama, A., Bachevalier, J., & Davis, M. (2012). Impaired safety signal learning may be a biomarker of PTSD. *Neuropharmacology, 62*(2), 695-704. doi:10.1016/j.neuropharm.2011.02.023

Jovanovic, T., & Norrholm, S. D. (2011). Neural mechanisms of impaired fear inhibition in posttraumatic stress disorder. *Frontiers in Behavioral Neuroscience, 5*, 44. doi:10.3389/fnbeh.2011.00044

Jovanovic, T., Norrholm, S. D., Fennell, J. E., Keyes, M., Fiallos, A. M., Myers, K. M., . . . Duncan, E. J. (2009). Posttraumatic stress disorder may be associated with impaired fear inhibition: Relation to symptom severity. *Psychiatry Research, 167*(1-2), 151-160. doi:10.1016/j.psychres.2007.12.014

Jovanovic, T., Nylocks, K. M., Gamwell, K. L., Smith, A., Davis, T. A., Norrholm, S. D., & Bradley, B. (2014). Development of fear acquisition and extinction in children: effects of age and anxiety. *Neurobiology of Learning and Memory, 113*, 135-142. doi:10.1016/j.nlm.2013.10.016

Jovanovic, T., Phifer, J. E., Sicking, K., Weiss, T., Norrholm, S. D., Bradley, B., & Ressler, K. J. (2011). Cortisol suppression by dexamethasone reduces exaggerated fear responses in posttraumatic stress disorder. *Psychoneuroendocrinology, 36*(10), 1540-1552. doi:10.1016/j.psyneuen.2011.04.008

Lommen, M. J. J., Engelhard, I. M., Sijbrandij, M., van den Hout, M. A., & Hermans, D. (2013). Pre-trauma individual differences in extinction learning predict posttraumatic stress. *Behaviour Research and Therapy, 51*(2), 63-67. doi:10.1016/j.brat.2012.11.004

Lovibond, S. H., & Lovibond, P. F. (1995). *Manual for the Depression Anxiety Stress Scales* (2nd ed.). Sydney: Psychology Foundation.

Merz, C. J., Hermann, A., Stark, R., & Wolf, O. T. (2014). Cortisol modifies extinction learning of recently acquired fear in men. *Social Cognitive & Affective Neuroscience, 9*(9), 1426-1434. doi:10.1093/scan/nst137

Meuret, A. E., Rosenfield, D., Bhaskara, L., Auchus, R., Liberzon, I., Ritz, T., & Abelson, J. L. (2016). Timing matters: Endogenous cortisol mediates benefits from early-day psychotherapy. *Psychoneuroendocrinology, 74*, 197-202. doi:10.1016/j.psyneuen.2016.09.008

Meuret, A. E., Trueba, A. F., Abelson, J. L., Liberzon, I., Auchus, R., Bhaskara, L., . . . Rosenfield, D. (2015). High cortisol awakening response and cortisol levels moderate exposure-based psychotherapy success. *Psychoneuroendocrinology, 51*, 331-340. doi:10.1016/j.psyneuen.2014.10.008

Milad, M. R., Orr, S. P., Lasko, N. B., Chang, Y., Rauch, S. L., & Pitman, R. K. (2008). Presence and acquired origin of reduced recall for fear extinction in PTSD: Results of a twin study. *Journal of Psychiatric Research, 42*(7), 515-520. doi:10.1016/j.jpsychires.2008.01.017

Milad, M. R., Orr, S. P., Pitman, R. K., & Rauch, S. L. (2005). Context modulation of memory for fear extinction in humans. *Psychophysiology, 42*(4), 456-464. doi:10.1111/j.1469-8986.2005.00302.x

Milad, M. R., Pitman, R. K., Ellis, C. B., Gold, A. L., Shin, L. M., Lasko, N. B., . . . Rauch, S. L. (2009). Neurobiological basis of failure to recall extinction memory in

posttraumatic stress disorder. *Biological Psychiatry, 66*(12), 1075-1082. doi:10.1016/j.biopsych.2009.06.026

Milad, M. R., Rauch, S. L., Pitman, R. K., & Quirk, G. J. (2006). Fear extinction in rats: Implications for human brain imaging and anxiety disorders. *Biological Psychology, 73*(1), 61-71. doi:10.1016/j.biopsycho.2006.01.008

Milad, M. R., Wright, C. I., Orr, S. P., Pitman, R. K., Quirk, G. J., & Rauch, S. L. (2007). Recall of fear extinction in humans activates the ventromedial prefrontal cortex and hippocampus in concert. *Biological Psychiatry, 62*(5), 446-454. doi:10.1016/j.biopsych.2006.10.011

Miller, G. E., Chen, E., & Zhou, E. S. (2007). If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans. *Psychological Bulletin, 133*(1), 25-45. doi:10.1037/0033-2909.133.1.25

Mineka, S., & Oehlberg, K. (2008). The relevance of recent developments in classical conditioning to understanding the etiology and maintenance of anxiety disorders. *Acta Psychologica, 127*(3), 567-580. doi:10.1016/j.actpsy.2007.11.007

Mørkved, N., Hartmann, K., Aarsheim, L. M., Holen, D., Milde, A. M., Bomyea, J., & Thorp, S. R. (2014). A comparison of Narrative Exposure Therapy and Prolonged Exposure therapy for PTSD. *Clinical Psychology Review, 34*(6), 453-467. doi:10.1016/j.cpr.2014.06.005

Morris, M. C., Compas, B. E., & Garber, J. (2012). Relations among posttraumatic stress disorder, comorbid major depression, and HPA function: a systematic review and meta-analysis. *Clinical Psychology Review, 32*(4), 301-315. doi:10.1016/j.cpr.2012.02.002

National Center for Posttraumatic Stress Disorder. (n.d.). Using the PTSD Checklist for DSM-IV (PCL). Retrieved from http://www.ptsd.va.gov/professional/pages/assessments/assessment-pdf/PCL-handout.pdf

Norrholm, S. D., Glover, E. M., Stevens, J. S., Fani, N., Galatzer-Levy, I. R., Bradley, B., . . . Jovanovic, T. (2015). Fear load: The psychophysiological over-expression of fear as an intermediate phenotype associated with trauma reactions. *International Journal of Psychophysiology, 98*(2 Pt 2), 270-275. doi:10.1016/j.ijpsycho.2014.11.005

Norrholm, S. D., Jovanovic, T., Olin, I. W., Sands, L. A., Karapanou, I., Bradley, B., & Ressler, K. J. (2011). Fear extinction in traumatized civilians with posttraumatic stress disorder: Relation to symptom severity. *Biological Psychiatry, 69*(6), 556-563. doi:10.1016/j.biopsych.2010.09.013

Orr, S. P., Lasko, N. B., Macklin, M. L., Pineles, S. L., Chang, Y., & Pitman, R. K. (2012). Predicting post-trauma stress symptoms from pre-trauma psychophysiologic reactivity, personality traits and measures of psychopathology. *Biology of Mood & Anxiety Disorders, 2*(8).

Orr, S. P., Metzger, L. J., Lasko, N. B., Macklin, M. L., Peri, T., & Pitman, R. K. (2000). De novo conditioning in trauma-exposed individuals with and without posttraumatic stress disorder. *Journal of Abnormal Psychology, 109*(2), 290-298. doi:10.1037/0021-843x.109.2.290

Pace-Schott, E. F., Spencer, R. M. C., Vijayakumar, S., Ahmed, N. A. K., Verga, P. W., Orr, S. P., . . . Milad, M. R. (2013). Extinction of conditioned fear is better learned and recalled in the morning than in the evening. *Journal of Psychiatric Research, 47*(11), 1776-1784. doi:10.1016/j.jpsychires.2013.07.027

Peri, T., Ben-Shakhar, G., Orr, S. P., & Shalev, A. Y. (2000). Psychophysiologic assessment of aversive conditioning in posttraumatic stress disorder. *Biological Psychiatry, 47*(6), 512-519. doi:10.1016/s0006-3223(99)00144-4

Pitman, R. K., Rasmusson, A. M., Koenen, K. C., Shin, L. M., Orr, S. P., Gilbertson, M. W., . . . Liberzon, I. (2012). Biological studies of post-traumatic stress disorder. *Nature, 13*, 769-787. doi:10.1038/nm3339

Powers, M. B., Halpern, J. M., Ferenschak, M. P., Gillihan, S. J., & Foa, E. B. (2010). A meta-analytic review of prolonged exposure for posttraumatic stress disorder. *Clinical Psychology Review, 30*(6), 635-641. doi:10.1016/j.cpr.2010.04.007

Raio, C. M., Brignoni-Perez, E., Goldman, R., & Phelps, E. A. (2014). Acute stress impairs the retrieval of extinction memory in humans. *Neurobiology of Learning and Memory, 112*, 212-221. doi:10.1016/j.nlm.2014.01.015

Roozendaal, B., Williams, C. L., & McGaugh, J. L. (1999). Glucocorticoid receptor activation in the rat nucleus of the solitary tract facilitates memory consolidation: involvement of the basolateral amygdala. *European Journal of Neuroscience, 11*(4), 1317-1323.

Rothbaum, B. O., & Davis, M. (2003). Applying learning principles to the treatment of post-trauma reactions. *Annals of the New York Academy of Sciemces, 1008*, 112-121.

Soravia, L. M., Heinrichs, M., Aerni, A., Maroni, C., Schelling, G., Ehlert, U., . . . de Quervain, D. J. (2006). Glucocorticoids reduce phobic fear in humans. *Proceedings of the National Academy of Science of the United States of America, 103*(14), 5585-5590. doi:10.1073/pnas.0509184103

Soravia, L. M., Heinrichs, M., Winzeler, L., Fisler, M., Schmitt, W., Horn, H., . . . de Quervain, D. J. (2014). Glucocorticoids enhance in vivo exposure-based therapy of spider phobia. *Depression and Anxiety, 31*(5), 429-435. doi:10.1002/da.22219

Vrana, S. R., & Lauterbach, D. (1994). Prevalence of traumatic events and post-traumatic psychological symptoms in a nonclinical sample of college students. *Journal of Traumatic Stress, 7*, 289-302.

Weathers, F. W., Litz, B. T., Huska, J. A., & Keane, T. M. (1994). *The PTSD Checklist-Civilian Version (PCL-C) for DSM-IV*. Boston: National Center for PTSD, Behavioral Sciences Division.

Yang, Y. L., Chao, P. K., & Lu, K. T. (2006). Systemic and intra-amygdala administration of glucocorticoid agonist and antagonist modulate extinction of conditioned fear. *Neuropsychopharmacology, 31*(5), 912-924. doi:10.1038/sj.npp.1300899

Yang, Y. L., Chao, P. K., Ro, L. S., Wo, Y. Y., & Lu, K. T. (2007). Glutamate NMDA receptors within the amygdala participate in the modulatory effect of glucocorticoids on extinction of conditioned fear in rats. *Neuropsychopharmacology, 32*(5), 1042-1051. doi:10.1038/sj.npp.1301215

Yehuda, R. (1997). Sensitization of the hypothalamic-pituitary-adrenal axis in posttraumatic stress disorder. *Annals of the New York Academy of Sciences, 821*, 57-75.

Yehuda, R. (2009). Status of glucocorticoid alterations in post-traumatic stress disorder. *Annals of the New York Academy of Sciences, 1179,* 56-69. doi:10.1111/j.1749-6632.2009.04979.x

Yehuda, R., Bierer, L. M., Pratchett, L. C., Lehrner, A., Koch, E. C., Van Manen, J. A., . . . Hildebrandt, T. (2015). Cortisol augmentation of a psychological treatment for warfighters with posttraumatic stress disorder: Randomized trial showing improved treatment retention and outcome. *Psychoneuroendocrinology, 51*, 589-597. doi:10.1016/j.psyneuen.2014.08.004

Yehuda, R., Giller, E. L., Southwick, S. M., Lowy, M. T., & Mason, J. W. (1991). Hypothalamic-pituitary-adrenal dysfunction in posttraumatic stress disorder. *Biological Psychiatry, 30*(10), 1031-1048.

Yehuda, R., Southwick, S. M., Krystal, J. H., Bremner, D., Charney, D. S., & Mason, J. W. (1993). Enhanced suppression of cortisol following dexamethasone administration in posttraumatic stress disorder. *The American Journal of Psychiatry, 150*(1), 83-86. doi:10.1176/ajp.150.1.83

Zuj, D. V., Palmer, M. A., Hsu, C. K., Nicholson, E. L., Cushing, P. J., Gray, K. E., & Felmingham, K. L. (2016). Impaired fear extinction associated with PTSD increases with hours-since-waking. *Depression and Anxiety, 33*(3), 203-210. doi:10.1002/da.22463

Zuj, D. V., Palmer, M. A., Lommen, M. J. J., & Felmingham, K. L. (2016). The centrality of fear extinction in linking risk factors to PTSD: A narrative review. *Neuroscience & Biobehavioral Reviews, 69*, 15-35. doi:10.1016/j.neubiorev.2016.07.014

Chapter 7

General Discussion

7.1 Overview of Thesis Aims and Outcomes

Impaired fear extinction learning and memory are considered primary influences of PTSD development and symptom persistence. The aim of the current thesis was to investigate key variables that may moderate the relationship between fear extinction and PTSD symptom severity. Chapter two presents a comprehensive narrative review of the central role that fear extinction appears to play in PTSD symptoms, as evidenced by shared relationships with risk factors for PTSD. Importantly, PTSD and fear extinction share the same neural networks of the amygdala, prefrontal cortex, and hippocampus, suggesting that biological and cognitive processes that rely on these networks are also involved. On balance, the literature consistently shows that impaired fear extinction is associated with, (1) the same polymorphisms of candidate genotypes involved in PTSD; (2) neuroendocrine and noradrenergic abnormalities associated with PTSD symptom expression; (3) similar effects of sex hormones estrogen and progesterone, potentially explaining sex differences in PTSD; (4) significant impacts of verbal functioning, which is considered primary complaint of PTSD; and (5) sleep disturbances and the negative impact of poor sleep quality. As fear extinction is considered to be a key component in the development of fear-related features of PTSD, and fear extinction has been shown to be associated with these risk factors, the aim of the current thesis was to explore some of these risk factors as potential moderators between fear extinction learning and PTSD symptoms. That is, the literature is currently unclear on whether fear extinction interacts with these risk factors in a model of PTSD symptoms. Due to sample size limitations, some of these risk factors (such as candidate genotypes and sex hormones) could not be included in the current thesis, and future research would benefit from investigating these relationships. This thesis presents a novel investigation of time-of-day, negative cognitive appraisals, noradrenaline, and cortisol as moderators of the relationship between fear extinction learning and PTSD symptoms.

The four empirical studies included in the current thesis (spanning chapters 3 – 6) present investigations of key variables (specifically, hours-since-waking, negative appraisals, noradrenaline, and cortisol, respectively) as moderators between fear extinction learning and PTSD symptoms. These studies used a cross-sectional clinical sample of participants with PTSD, trauma-exposure without PTSD, and non-trauma-exposed healthy controls. Skin conductance served as the primary index of fear conditioned responses, and stress hormone levels were assessed via saliva assays for cortisol and salivary alpha-amylase (as a proxy for noradrenergic functioning). Figure 1 presents a graphical representation of the primary empirical findings from the current thesis. Subsequent sections summarize the key findings and implications of each chapter, followed by theoretical implications, methodological limitations, and directions for future research.

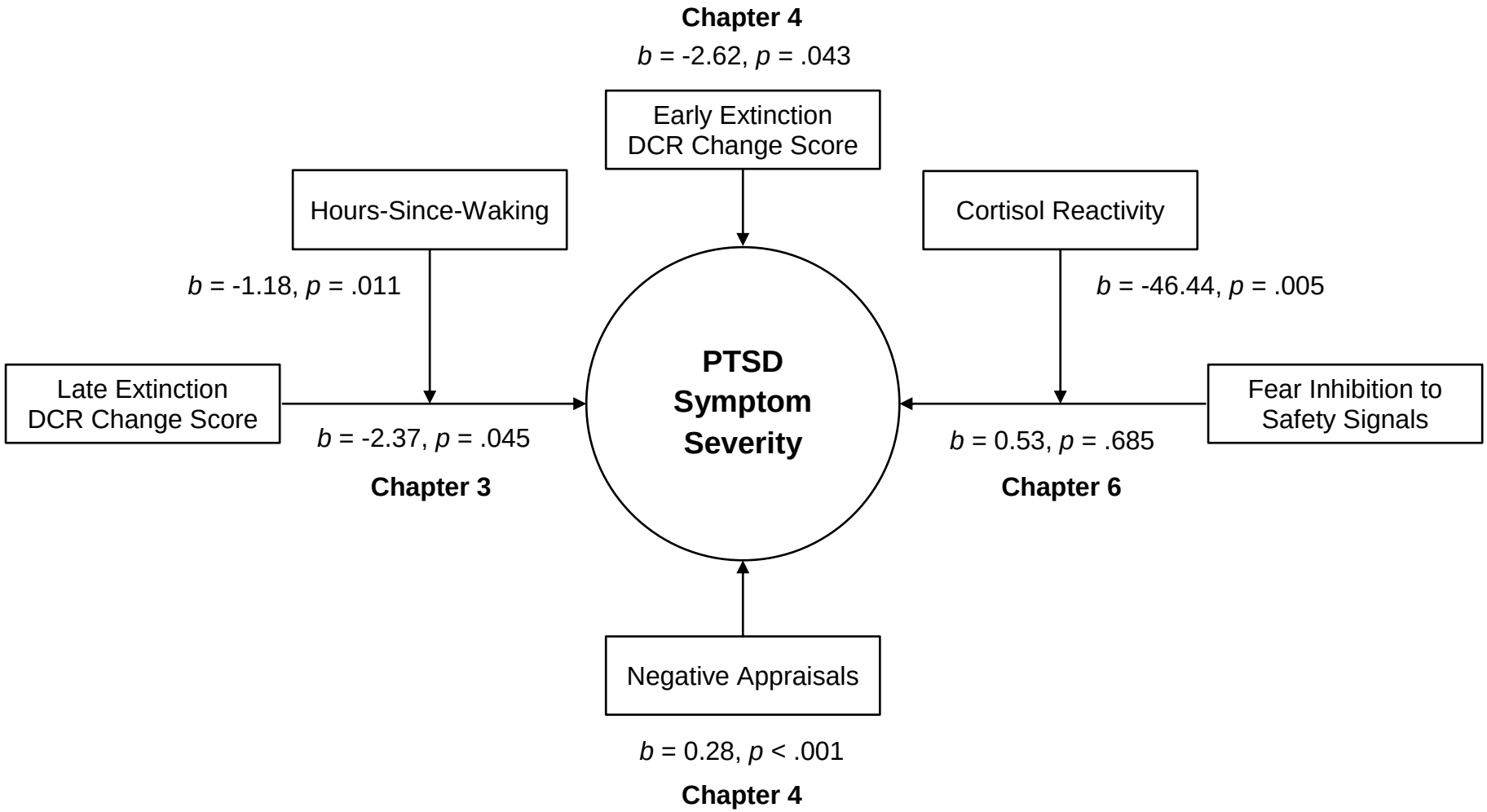

Figure 1. Significant direct effects and moderation interactions from Chapters 3, 4, and 6. This figure displays a conceptual diagram of the interactions between predictors and moderators on PTSD symptom severity, and is not a statistical representation of relationships (Hayes, 2013).

7.2 Key Empirical Findings and Implications of the Thesis

7.2.1 The role of sleep in fear extinction (Chapter 3).

Sleep disturbances are widely considered a hallmark symptom of PTSD (Germain, 2013; Ross, Ball, Sullivan, & Caroff, 1989), and poor sleep can adversely impact cognitive and emotional functioning. Further, sleep plays a key role in the consolidation of emotional memories (Pace-Schott, Germain, & Milad, 2015), including fear extinction memories (Spoormaker et al., 2010). Time-of-day has recently been found to be an important factor in fear extinction memories, with significantly better extinction learning and memory reported in the morning compared to the evening in healthy participants (Pace-Schott et al., 2013). Chapter three examined whether the relationship between fear extinction learning and PTSD symptoms changes as a factor of hours-since-waking. Moderation analyses revealed a significant interaction between fear extinction learning and hours-since-waking in predicting PTSD symptom severity. Specifically, participants with higher PTSD symptoms showed significantly poorer fear extinction learning, and this linear relationship became stronger as participants were awake for longer.

A limitation of the study presented in Chapter three is the absence of a polysomnographic-recording of sleep wave architecture. For example, as reviewed in Chapter two, previous research in rodents and humans has shown deprivations in rapid eye movement (REM) sleep to significantly impair fear extinction learning (Silvestri & Root, 2008) and memory (Fu et al., 2007; Pace-Schott, Verga, Bennett, & Spencer, 2012; Spoormaker et al., 2012). Rather, Chapter three used hours-since-waking as a measure of homeostatic sleep pressure, which refers to the notion that cognitive and emotional performance are at their best soon after waking, and slowly become worse throughout the day as an individual's need for sleep increases. It was recently argued that extinction learning deficits later in the day can be attributed to homeostatic sleep pressures (Pace-Schott et al., 2014), whereby increasing

adenosine activity in the basal forebrain correlate with sleep deprivation to promote a greater need for sleep (Porkka-Heiskanen & Kalinchuk, 2011). In other words, we argue that extinction capacity is enhanced due to the restorative benefits of sleep. Should this interpretation hold true, these findings carry important implications for the implementation of treatment, suggesting that exposure therapy may be most beneficial when scheduled soon after waking, rather than later (Pace-Schott et al., 2013; Pace-Schott et al., 2014; Zuj, Palmer, Hsu, et al., 2016).

Previous research shows alterations in cortisol output with circadian rhythm (Kalsbeek et al., 2012). Further, Pace-Schott et al. (2013) found that higher testosterone/cortisol ratio was associated with better fear extinction learning in the morning. To examine whether cortisol had any effect on hours-since-waking as a moderator between fear extinction and PTSD symptoms, basal cortisol, post-acquisition cortisol, and cortisol reactivity were individually tested as covariates of this relationship (see section 3.8 of the current thesis). Moderation analyses revealed that cortisol was not a significant covariate, and all main effects and interactions remained the same. Nevertheless, increased cortisol has previously been shown to enhance therapeutic extinction (Soravia et al., 2006; Soravia et al., 2014), and should be taken into consideration in future studies of time-of-day effects on extinction and PTSD symptoms.

7.2.2 The role of cognition in fear extinction (Chapter 4).

The cognitive theory of PTSD (Ehlers & Clark, 2000) proposes that PTSD symptoms persist due to excessive negative appraisals about the trauma and its sequelae. Evidence suggests that the cognitive theory of PTSD and the fear extinction account are not mutually exclusive, as conditioned fear responses triggered by trauma reminders are thought to reinforce negative appraisals (Ehlers & Clark, 2000). To our knowledge, however, no

previous studies have examined fear extinction and negative appraisals in tandem, in relation to PTSD. Chapter four investigated negative appraisals as a moderator of the relationship between fear extinction learning and PTSD symptoms. The moderation analysis revealed excessively negative appraisals and poor fear extinction learning ability were significantly associated with greater PTSD symptom severity. Despite this, however, there was no significant moderation between negative appraisals and fear extinction, suggesting that these two factors may function independently in relation to PTSD. While this study was conducted in an experimental framework, the findings presented in Chapter four highlight that there are both cognitive and biological processes in PTSD which may benefit from targeted treatment approaches. That is, should this notion hold true, these findings suggest the need for exposure therapy to promote fear extinction, as well as cognitive therapy targeting catastrophic negative appraisals.

Previous research has demonstrated that imaginal exposure therapy combined with cognitive restructuring (aimed at correcting negative appraisals of the trauma) resulted in significantly enhanced treatment outcome compared to imaginal exposure alone (Bryant, Moulds, Guthrie, Dang, & Nixon, 2003). This finding is in contrast to conclusions that prolonged exposure therapy combined with cognitive restructuring has no additive benefits over prolonged exposure therapy alone (Foa et al., 2005; Marks, Lovell, Noshirvani, Livanou, & Thrasher, 1998). While the research presented in Chapter four was not conducted in a clinical treatment setting, the results suggest that fear extinction learning and catastrophic negative appraisals do not interact, and show independent relationships with PTSD symptoms, perhaps requiring specific attention during treatment.

Regarding cognition and PTSD more generally, consistent research has found cognitive performance deficits in PTSD, particularly in verbal learning and memory (e.g., Barrett, Green, Morris, Giles, & Croft, 1996; Bremner, Vermetten, Afzal, & Vythilingam,

2004; Gilbertson et al., 2006; Grigorovich, Gomez, Leach, & Fish, 2013). The effects of verbal functioning on fear extinction learning ability, however, have received little attention. Of the few studies conducted to assess this, one study found that engaging with a verbal worrying task led to poorer fear extinction learning (Gazendam & Kindt, 2012), providing preliminary evidence that fear extinction is not an automatic process, but relies on cognitive resources (Raes, De Raedt, Verschuere, & De Houwer, 2009). Indeed, greater cognitive demand results in weaker fear conditioning (Carter, Hofstotter, Tsuchiya, & Koch, 2003), however the effects on extinction are unclear.

As opposed to research in rodents, human fear extinction learning appears to be modulated by cognitive processes (Hermans, Craske, Mineka, & Lovibond, 2006; P. F. Lovibond, 2004). That is, any disparities found between research in animals and humans is argued to be due to higher-order cognitive processing in humans, resulting in the ability to verbally and symbolically understand US contingencies (Hermans et al., 2006). This higher-order understanding of conditioning is reflected in a study showing similar changes in SC responding and US-expectancy ratings to CS and safety signals, suggesting the convergence of conditioned SCR and cognitive threat expectancy (P. F. Lovibond, 2004; P. F. Lovibond, Davis, & O'Flaherty, 2000). In summary, fear extinction in humans appears to be driven by key cognitive processes (P. F. Lovibond, 2004), and the absence of available cognitive resources can have significant consequences for extinction performance (Raes et al., 2009).

7.2.3 The role of stress hormones in fear extinction (Chapters 5 and 6).

The stress response involves two waves of neurochemical release (Joëls & Baram, 2009). The first wave of the stress response involves the rapid release of adrenaline and noradrenaline in sympathetic arousal structures to respond to an immediate stressor. The second wave of the stress response involves the hypothalamic-pituitary-adrenal (HPA) axis,

and its end product, cortisol, to shut down the stress response. The key finding of Chapter five suggests that noradrenaline is not a significant moderator between fear extinction and PTSD, while Chapter six shows cortisol reactivity to be a significant moderator between fear inhibition to safety signals and PTSD symptoms.

Evidence suggests that a cortisol deficit may be involved in the etiology of PTSD (Pitman et al., 2012), with cortisol administration to cardiac surgery patients reducing posttraumatic stress symptoms (Schelling et al., 2004). Recent evidence suggests that reducing current situational fear expression of patients receiving exposure therapy may be an effective means of reducing patient drop-out rates, leading to greater treatment outcomes (Yehuda et al., 2015). Indeed, the findings of Chapter six show that enhanced fear extinction learning ability is associated with lower PTSD symptoms, and this relationship becomes stronger with elevated cortisol reactivity. Further, this finding was only in relation to the CS-, and not the CS+, potentially reflecting cortisol influences on the processes of fear inhibition, which is gaining attention as a key biomarker of PTSD symptoms (Jovanovic et al., 2012; Jovanovic & Norrholm, 2011). Essentially, fear expression in safe contexts or to stimuli signaling safety may be due, in part, to cortisol reactivity. Enhancing cortisol release may facilitate corrective fear expression to safe contexts, and these findings are in line with recent clinical evidence that elevating cortisol leads to greater exposure therapy response in PTSD (Yehuda et al., 2015).

Cortisol output is also modulated by the menstrual cycle, as increased estrogen attenuates stress-related activity in the HPA axis and noradrenergic system (Charney, 2004). There has been a growing research interest in the effect of sex hormones on fear extinction learning and PTSD symptoms. Consistent research in healthy controls shows that lower estrogen is associated with impaired fear extinction learning (Wegerer, Kerschbaum, Blechert, & Wilhelm, 2014) and recall (Milad et al., 2010; Zeidan et al., 2011). Participants

with PTSD have shown convergent evidence, with low estradiol associated with impaired fear extinction (Glover et al., 2012) and inhibition (Glover et al., 2013). In contrast, a recent study demonstrated that women with PTSD showed poorer fear extinction retention in the midluteal phase of the menstrual cycle, which was attributed to elevated progesterone levels (Pineles et al., 2016). These findings suggest that estrogen and progesterone may also be important moderators of the relationship between fear extinction and PTSD symptoms, and future research using moderation (or mediation) may reveal direct and/or indirect effects between these variables.

7.3 Theoretical Implications

A deeper understanding of the behavioral and biological factors that shape the role of fear extinction in PTSD would allow for more targeted treatment methods (Holmes & Singewald, 2013). As noted by Mineka and Oehlberg (2008), the classical conditioning and extinction approach to PTSD has previously been criticized for being too simplistic in explaining the development and maintenance of symptoms for such disorders as PTSD. As reviewed in Chapter two, a number of PTSD-related risk factors have been identified that also share relationships with fear extinction learning (Zuj, Palmer, Lommen, & Felmingham, 2016). This narrative review, and the increasing evidence of key moderators of fear extinction learning, suggests that current fear extinction models need to incorporate broader domains of biological and cognitive influences (Myers & Davis, 2007; Orsini & Maren, 2012). Further research should also examine the temporal relationships between these variables. That is, are some of these variables pre-trauma vulnerability factors? It is important to understand when and how stress hormones, sleep disturbances, and cognitions impact the trajectory from trauma to PTSD symptoms. Prospective longitudinal studies are required to answer these questions.

The study presented in Chapter four investigated the relationship between fear extinction learning and negative appraisals, a key aspect of the cognitive theory of PTSD (Ehlers & Clark, 2000). In particular, we confirmed predictions that both fear extinction learning and negative appraisals influence PTSD, but we did not support the hypothesis of an interaction between these two variables. A limitation of this study, however, is that this model was assessed in a lab-based setting, with non-trauma-relevant conditioned stimuli. Using a conditioning paradigm with trauma-relevant stimuli (e.g., see Wegerer et al., 2014) may produce stronger conditioned emotional responses, which could demonstrate a relationship with trauma-related negative appraisals. The cognitive model further hypothesizes that persistent PTSD is caused by fragmented and poorly contextualized trauma memories, and dysfunctional behavioral and cognitive strategies aimed at reducing feelings of threat, yet further exaggerating symptoms (Ehlers, Mayou, & Bryant, 2003). In relation to this, a limitation of Chapter four is that we were unable to examine these aspects of the model, and future research would benefit from investigating the role of extinction in the elaboration of autobiographical trauma memories, and the nature of intrusive memories in PTSD. A conditioned-intrusion paradigm, as used by Wegerer et al. (2014), may be useful here.

The findings of Chapter four show significant associations between negative appraisals and PTSD, and between impaired fear extinction learning and PTSD, but no moderation interaction was found. This finding suggests that these two theories each present important variables involved in PTSD symptoms, however these constructs are in parallel with each other, showing no interactions in relation to PTSD. This has important implications for the potential integration of theories of PTSD symptom development and maintenance. Specifically, there has been little convergence of theory and empirical literature, and more research is needed to examine potential links between cognitive and biological influences.

While the findings of Chapter four did not demonstrate clear links between the fear extinction account and the cognitive model of PTSD (Ehlers & Clark, 2000), future research should explore trauma-relevant stimuli in the context of fear conditioning and extinction paradigms with increased unpredictability of aversive stimuli, and record of instructive memories. Examining these effects on fear extinction recall as well as extinction learning may identify previously unexplored convergences of theory.

The notion that PTSD is associated with increased fear expression to safety signals is receiving increased interest. Impaired fear inhibition to safety signals is argued to be an intermediate phenotype between the neurocircuitry and clinical symptoms of PTSD (Jovanovic & Norrholm, 2011). Further, it has been argued that issues in differentiating threat versus safety signals is a reflection of impaired contextual processing in PTSD (for a review, see Liberzon & Abelson, 2016). The study findings presented in Chapter six showed that cortisol reactivity was a moderator between fear inhibition to a safety signal (but not the previously reinforced CS+) and PTSD symptoms. These findings provide support for the notion that PTSD is associated with heightened fear expression to a safety signal, and that this relationship appears to be shaped by cortisol reactivity.

7.4 Methodological Limitations

7.4.1 Cross-sectional study design.

The first and foremost limitation of the research in this thesis is that it was cross-sectional, rather than longitudinal. That is, causal interpretations are constrained and it remains unclear whether the variables being observed aided in the development of PTSD symptoms. For example, the findings presented in Chapter six suggest that cortisol reactivity shapes the role of fear extinction learning (in particular, fear to a safety signal) in PTSD. While this evidence presents some interesting and novel insights into the role of cortisol in

fear extinction in PTSD, it remains unclear whether this interaction plays a role in the development of conditioned fear responses that are resistant to extinction. For example, a prospective study by McFarlane, Barton, Yehuda, and Wittert (2011) assessed cortisol in the acute aftermath of a trauma, and found a negative correlation between morning cortisol and PTSD symptoms six-months later. Thus, longitudinal studies would allow for robust conclusions to be drawn on the specific role of fear extinction learning and memory in PTSD. As highlighted in Chapter two, there is considerable prospective evidence for a role of the variables presented in the current thesis (that is, sleep, cognitions, noradrenaline, and cortisol), however the temporal timeframe of these variables is unclear. Specifically, future research investigating the role that these variables (and impaired fear extinction) play in the trajectory of trauma to PTSD symptom development may reveal important insights for early detection and intervention.

7.4.2 Indices of conditioned fear responding.

The studies presented in the current thesis used SCR as the primary index of conditioned responding, and self-reported expectancy ratings of the unconditioned stimulus (US-expectancy) as a measure of threat expectancy. While SCR is a commonly used psychophysiological measure of conditioned responding (e.g., Blechert, Michael, Vriends, Margraf, & Wilhelm, 2007; Milad, Orr, Pitman, & Rauch, 2005; Orr et al., 2000; Pace-Schott et al., 2013), it suffers from limitations such as substantial artefact and individual variability, with upwards of 37% attrition rates reported due to participants classed as SCR non-responders (e.g., Inslicht et al., 2013; Pineles et al., 2016; Spring et al., 2015). Although SCR is considered a measure of sympathetic arousal (Boucsein, 1992), there is some evidence that SCR actually reflects cognitive threat expectancy (P. F. Lovibond, 2004) and anticipatory arousal irrespective of stimulus valence (Weike, Schupp, & Hamm, 2007). Alternatively,

many studies also implement electromyogram (EMG) recordings of acoustic startle (e.g., Guthrie & Bryant, 2006; Jovanovic et al., 2005; Norrholm et al., 2011), which reflects subcortical fear networks, particularly afferent and efferent amygdala pathways to the brainstem (Davis & Whalen, 2001; Kindt & Soeter, 2013). Although both psychophysiological methods have shown robust effects in differential fear conditioning and extinction, a longitudinal study by Guthrie and Bryant (2006) revealed that only EMG-recorded fear extinction impairments were a significant pre-trauma risk factor of PTSD symptoms in trainee firefighters. Replication of the studies in the current thesis (or failure to do so) using EMG-recorded acoustic startle would provide important insights into the role of subcortical arousal in these interactions.

Recent evidence in healthy participants suggests that conscious cognitive awareness of the CS-US contingency is necessary for fear conditioned SC responding, but not for conditioned startle data (Sevenster, Beckers, & Kindt, 2014). A strength of the current research is that self-report US-expectancy responses were obtained from participants alongside SCR throughout the fear conditioning and extinction paradigm. Participants who reported uncertain threat expectancy of the US (that is, US-expectancy ratings less than 60) during CS+ trials at the end of the fear acquisition phase were excluded from all analyses (Lommen, Engelhard, Sijbrandij, van den Hout, & Hermans, 2013). Nevertheless, future research would benefit from the inclusion of multiple physiological indices of conditioned fear responding for cross-validation (Sevenster et al., 2014).

The current program of research also showed discrepancies in the findings of SCR amplitude data, compared to self-report US-expectancy ratings. SCR amplitude data revealed significant group effects mainly in early extinction (Chapters 4-6), whereas group effects for US-expectancy data were limited to the late extinction phase. Specifically, the PTSD group displayed significantly greater threat expectancy during late extinction compared to the TC

and NTC groups, and this effect did not change as a factor of CS-type or trial. Despite this discrepancy, US-expectancy methods have been found to be an externally valid measure of human fear conditioning (Boddez et al., 2013). Although recent evidence suggests that CS-US contingency awareness is necessary for fear conditioned SC responses (Sevenster et al., 2014), Blechert and colleagues (2008) found that fear extinction occurs rapidly in SCR amplitude data, and slower in US-expectancy ratings. This pattern of effects across SCR and US-expectancy ratings might reflect more rapid psychophysiological responding despite sustained threat processing, reinforcing a need for targeted cognitive threat expectancy.

7.4.3 Discrepant findings.

Due to between-group discrepancies in fear conditioning and extinction across the empirical chapters, moderation analyses included different indices of extinction learning. For example, Chapter three used a differential conditioned response (DCR) change score for the late extinction phase, while Chapters four and five used a DCR change score for the early extinction phase. DCR change scores provide an index of the change in responding between the CS+ and CS- across the experimental phase, whereby larger scores in extinction represent a greater decline in differentiation between the threat and safety signal (that is, better extinction learning). Alternatively, the extinction index used in moderation analyses for Chapter six involved a change score between trials 1 and 2 of early extinction, calculated separately for the CS+ and CS-. This change score was calculated as the pattern of effects for early extinction reflected rapid attenuation of SC responses from trial 1 to trial 2 for the TC and NTC groups, yet this change was not significant for the PTSD group. By trial 3, all groups were displaying comparative levels of fear responses. These respective extinction learning indices were calculated to reflect the nature of group effects in fear extinction for those chapters, and moderators were used in an attempt to explain relevant effects. The

extinction index used in Chapter six also provides useful information on conditioned responses to threat versus safety signals. A limitation of DCR change scores is that you cannot evaluate individual responding to the threat signal, as opposed to the safety signal. This is of particular importance due to the recent increase in research that emphasizes the importance of fear responding to safety signals (Jovanovic, Kazama, Bachevalier, & Davis, 2012; Jovanovic & Norrholm, 2011; Jovanovic et al., 2010; Jovanovic & Ressler, 2010).

7.4.4 Unconditioned stimulus reinforcement schedule.

The studies reported in the current thesis used a 100% reinforcement schedule, meaning that every presentation of the CS+ was reinforced by the US in the acquisition phase. At current, the field appears to be largely mixed, with some studies using partial reinforcement schedules (60-62.5%; e.g., Milad et al., 2009; Pace-Schott et al., 2013) and others using full reinforcement schedules (e.g., Jovanovic et al., 2014; Orr et al., 2012; Orr et al., 2000), with largely convergent findings between these studies. Whilst studies have examined the impact of full versus partial reinforcement schedules in healthy controls (Grady, Bowen, Hyde, Totsch, & Knight, 2016), no studies have examined the impact of full versus partial reinforcement in PTSD. Full reinforcement schedules in healthy controls, however, appear to result in rapid extinction learning (Grady et al., 2016), suggesting that reinforcement schedules of less predictable threat may enhance the sensitivity to identify effects. Interestingly, a partial reinforcement schedule followed by full reinforcement showed strong conditioned responding and prolonged extinction (Grady et al., 2016), suggesting that this US reinforcement schedule may be useful for future research.

Altering the reinforcement schedule during fear acquisition results in changes to the prediction error of the US. The prediction error signal refers to the discrepancy between expected and actual outcomes during fear learning (Li & McNally, 2014). Importantly, when

there are no errors in prediction and the expected outcome is encountered upon every trial (for example, such as the full reinforcement schedule used in the current research), the "US is not surprising and no increments in fear learning occur" (Li & McNally, 2014, p. 15). Increasing the prediction error (for example, implementing a partial reinforcement rate) enhances the unpredictability of the US, thus producing small increases in fear learning that may be more resistant to extinction. Rapid extinction learning, as seen in the current program of research, is likely due to the absence of prediction error during the acquisition phase, as the CS has never been presented in situations of uncertainty. In other words, participants may come to expect that during each given experimental phase, the US will either be encountered following all CS+ presentations, or none of them. Research shows that increasing US-reinforcement ambiguity early in conditioning, followed by full reinforcement produces stronger fear acquisition and slower extinction learning (Grady et al., 2016).

7.4.5 Early to late extinction SCR uptick.

The fear conditioning and extinction paradigm used in the current program of research involved a small 1-minute interval between the early and late extinction phases (as in Milad et al., 2005). During this interval, a prompt was displayed on the computer screen instructing participants to continue when ready. As seen in the graphical representations of fear conditioning and extinction results throughout this thesis, there is an uptick in SCRs from the last trial of early extinction to the first trial of late extinction for both the CS+ and the CS-. It is possible that this is a form of short-term spontaneous recovery. Previous research has examined reinstatement immediately following fear extinction in order to avoid any spontaneous recovery effects (Norrholm et al., 2006). Further, there is some uncertainty regarding the temporal difference between acquisition and extinction phases before spontaneous recovery might occur (Norrholm et al., 2008), suggesting this might explain the

uptick in SCRs between early and late extinction. An alternative explanation is that due to the on-screen prompt between early and late extinction, participants may be under the impression that the CS+/US contingency may have changed and that further mild electric shocks may occur. Future research would benefit from using a continuous fear extinction phase, which can be split during data entry for analysis purposes.

7.4.6 Sample size and PTSD classification.

The PTSD Checklist-Civilian version (PCL-C; Weathers, Litz, Huska, & Keane, 1994) is a 17-item self-report questionnaire that corresponds with the diagnostic criteria of the DSM-IV (American Psychiatric Association, 2000), and was used as the diagnostic instrument for PTSD in the current thesis. Diagnostic criteria for the DSM-IV were used as data collection began prior to diagnostic criteria for the DSM-V becoming available. The PCL-C provides diagnostic information, as well as an ordinal measure of symptom severity, with a score equal to, or greater than 50 representing clinically severe PTSD symptoms (National Center for Posttraumatic Stress Disorder, n.d.). While the majority of individuals classified as PTSD did meet diagnostic criteria for PTSD, some participants with subclinical symptoms (defined as having a PCL total score greater than 40; National Center for Posttraumatic Stress Disorder, n.d.) were included in the PTSD group to increase statistical power, but may have also weakened effects. Importantly, this would have had little impact on moderation analyses as an ordinal PCL total score was used as the primary outcome variable in these analyses. Future research would benefit from a larger sample size with exclusively clinical PTSD symptoms, in order to make robust conclusions on the relationship between fear extinction learning and clinical PTSD symptoms.

7.4.7 Sample characteristics.

PTSD is often comorbid with other psychiatric conditions, particularly depression. In the current program of research, an ordinal measure of depressive symptoms was obtained using the Depression Anxiety Stress Scale 21-item version (DASS; S. H. Lovibond & Lovibond, 1995). Where necessary, depression was included as a covariate and did not significantly alter the pattern of effects (for example, see section 3.8 of the current thesis). Including greater consideration of comorbidity may also have allowed conclusions to be drawn regarding the specific effects of PTSD compared to such comorbid disorders. Further, due to sample size restrictions and the variability of medications, these were not controlled in the current program of research. For example, medications such as oral contraceptives have been shown to temporarily impair fear extinction learning (Graham & Milad, 2013), and were not controlled for in the current thesis. Taking these factors into account with larger sample sizes will strengthen future research.

7.5 Directions for Future Research

7.5.1 Impairments in fear extinction memory.

The research presented in the current thesis examined the relationships between key factors and fear extinction learning in PTSD. Previous research indicates that many individuals with PTSD do not show impairments in extinction learning ability (Milad et al., 2008; Milad et al., 2009), but show deficits in the memory for fear extinction (Milad et al., 2008; Milad et al., 2009; Shvil et al., 2014). That is, fear extinction may be learned effectively, but poorly recalled the following day. Further, the fear extinction account of PTSD proposes extinction as constituting a new memory trace that competes with the original fear acquisition trace (Myers & Davis, 2002). In some circumstances, the conditioning memory will override the extinction memory, resulting in the return of conditioned fear

expression (see Figure 2). We may expect to see stronger effects in fear extinction recall, as both sleep (Pace-Schott et al., 2015) and cortisol (Zoladz & Diamond, 2013) have notable effects on memory consolidation processes. Further, risk factors in the present thesis that showed no interactions with fear extinction learning (that is, negative appraisals and noradrenaline), may instead be associated with the consolidation of fear extinction memories. Indeed, increased NA is associated with enhanced emotional learning and memory processes (Mueller & Cahill, 2010), suggesting that NA may play a prominent role in fear extinction memory, rather than learning. Thus, a two-day extinction learning and recall paradigm may reveal NA activity (and other potential moderators) to be a significant moderator between fear extinction recall and PTSD symptoms. These interactions may also be assessed in relation to a number of fear return concepts, such as spontaneous recovery, fear renewal, and reinstatement (see Figure 2). Examining risk factors discussed in the current thesis in relation to each of these concepts would lead to important insights into how fear extinction operates to influence fear-related disorders.

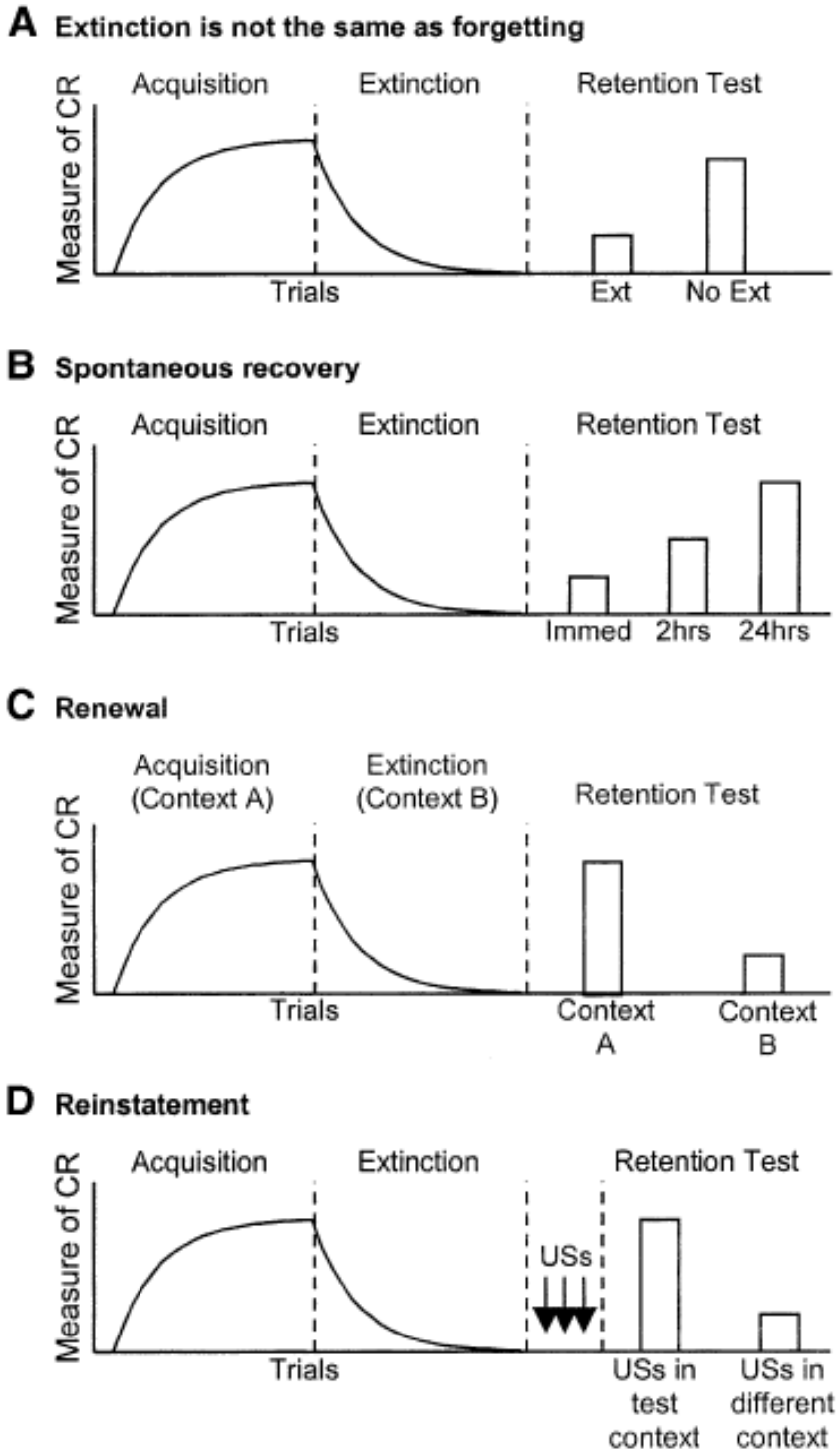

Figure 2. Models suggestive of fear extinction to constitute the formation of a new memory. Panel **(A)** shows that fear extinction forms a new memory, as the acquired fear trace remains until extinction is learned. Spontaneous recovery **(B)** demonstrates that as time increases post-extinction learning, the conditioned response can return in increased magnitude. Contextual fear extinction research **(C)** shows that the acquisition of fear in one context (i.e.,

context A) and extinction in a second context (i.e., context B) may allow ongoing conditioned fear responding in context A. Following successful fear extinction learning, re-experiencing the US in the absence of the CS may reinstate the conditioned fear response **(D)** (adapted from Myers & Davis, 2002, 2007).

7.5.2 The genetics of impaired fear extinction in PTSD.

As discussed in Chapter two, PTSD is associated with numerous biological, cognitive, and epigenetic features, some of which may be pre-trauma risk factors. Genetics is currently claimed to be one of the most promising fields of enhancing our understanding of PTSD risk and resilience (Zoladz & Diamond, 2013; Zuj, Palmer, Lommen, et al., 2016). For example, a candidate genotype discussed in Chapter two of the current thesis is FKBP5 (see section 2.9.1), which regulates cortisol output and glucocorticoid sensitivity (Bomyea, Risbrough, & Lang, 2012), and may be an important precursor to altered cortisol and the hypersensitivity of glucocorticoid receptors in PTSD. With particular relevance to the findings of Chapter six, the significant moderation interaction between cortisol reactivity and fear extinction to safety signals may be modulated by underlying effects of FKBP5 expression on the molecular networks of the HPA axis. Future research investigating this research question, and others related to the candidate genotypes reviewed in Chapter two, may show important effects of genetics on the relationship between fear extinction and PTSD.

7.5.3 Moderation versus mediation.

In the current program of research, moderation analyses were used to test hypotheses of the role of key variables in the relationship between fear extinction and PTSD symptom severity. Moderation is a regression-based analysis method, whereby the relationship

between a predictor variable and an outcome variable changes as a factor of the moderator (Hayes, 2013). That is, the predictor variable and moderator interact in their prediction of the outcome (see Figure 3A). An alternative statistical analysis method is mediation, whereby the predictor variable causes a change in the mediator, which in turn causes a change in the outcome variable (see Figure 3B). Mediation models are most useful when used in prospective longitudinal designs to determine the mechanism of change between a predictor variable (such as trauma exposure) and an outcome variable (such as PTSD symptoms). Importantly, the use of mediation analyses coupled with prospective longitudinal studies would be ideal for increasing our theoretical and clinical understanding of the temporal influence of risk factors in the trajectory from trauma exposure to PTSD symptoms.

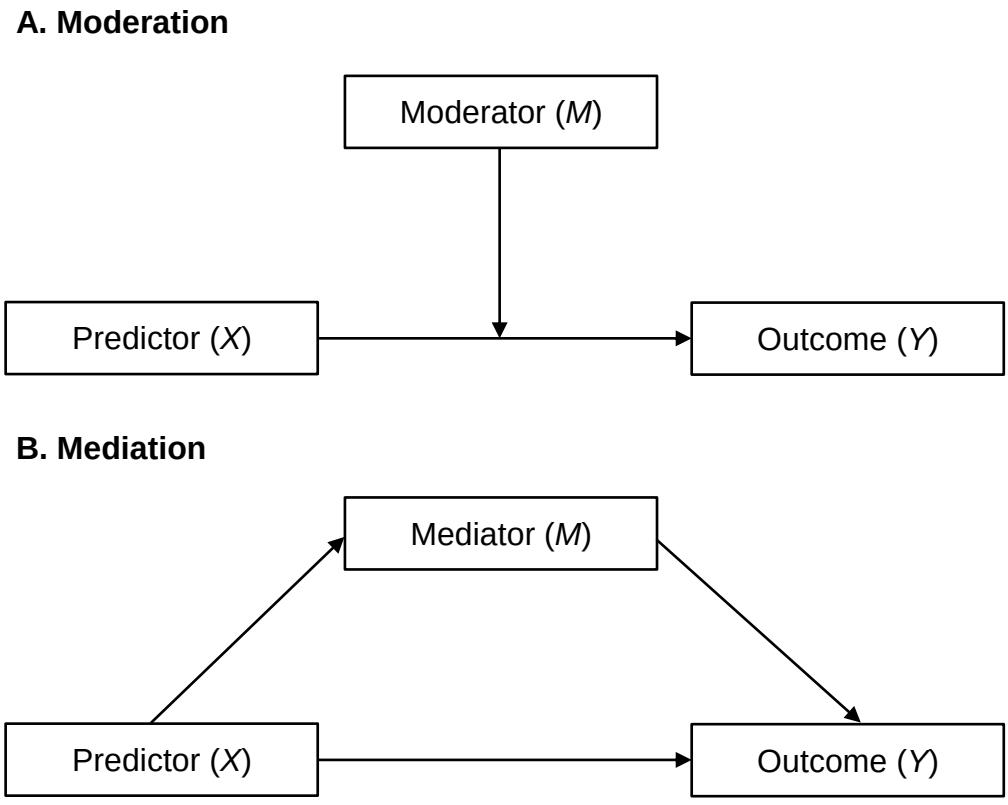

Figure 3. Conceptual graphical representation of moderation **(A)** and mediation **(B)** models (adapted from Hayes, 2013).

7.6 Conclusions

The aim of the current thesis was to investigate the complexity of fear extinction in PTSD, by assessing the additional factors that may moderate the relationship between fear extinction learning and PTSD. The narrative review presented in Chapter two shows a number of biological and cognitive factors that appear to link fear extinction learning and memory to PTSD, however empirical research in these areas is lacking. Collectively, the original research presented in this thesis indicates that the relationship between impaired fear extinction learning and PTSD symptoms is complex, and involves multiple moderating factors – including hours-since-waking and cortisol reactivity, not to mention the influence of negative appraisals on PTSD symptoms independently of impaired fear extinction. While preliminary, the findings presented herein have important implications for exposure-based treatment methods for PTSD. These implications may simply involve strategically scheduling treatment shortly after sleep, or pharmacologically augmenting cortisol activity to enhance treatment benefit. While the current thesis used a within-session conditioning and extinction learning paradigm, these findings present novel questions for future research regarding moderators of the consolidation of extinction memories, and how these effects might importantly translate to a clinical setting. A similar program of research using primarily mediation models would compliment the findings of the thesis and would shed light on a number of issues in modeling PTSD etiology, and our understanding of the mechanisms of this disorder.

7.7 References

American Psychiatric Association. (2000). *Diagnostic and Statistical Manual of Mental Disorders* (4th ed.). Washington, D.C: American Psychiatric Association.

Barrett, D. H., Green, M. L., Morris, R., Giles, W. H., & Croft, J. B. (1996). Cognitive functioning and posttraumatic stress disorder. *American Journal of Psychiatry, 153*(11), 1492-1494.

Blechert, J., Michael, T., Vriends, N., Margraf, J., & Wilhelm, F. H. (2007). Fear conditioning in posttraumatic stress disorder: Evidence for delayed extinction of autonomic, experiential, and behavioural responses. *Behaviour Research and Therapy, 45*(9), 2019-2033. doi:10.1016/j.brat.2007.02.012

Blechert, J., Michael, T., Williams, S. L., Purkis, H. M., & Wilhelm, F. H. (2008). When two paradigms meet: Does evaluative learning extinguish in differential fear conditioning? *Learning and Motivation, 39*(1), 58-70. doi:10.1016/j.lmot.2007.03.003

Boddez, Y., Baeyens, F., Luyten, L., Vansteenwegen, D., Hermans, D., & Beckers, T. (2013). Rating data are underrated: validity of US expectancy in human fear conditioning. *Journal of Behavior Therapy and Experimental Psychiatry, 44*(2), 201-206. doi:10.1016/j.jbtep.2012.08.003

Bomyea, J., Risbrough, V., & Lang, A. J. (2012). A consideration of select pre-trauma factors as key vulnerabilities in PTSD. *Clinical Psychology Review, 32*(7), 630-641. doi:10.1016/j.cpr.2012.06.008

Boucsein, W. (1992). Electrodermal activity. In W. J. Ray (Ed.), *Psychophysiology and Medicine* (pp. 1-420). New York: Plenum Press.

Bremner, J. D., Vermetten, E., Afzal, N., & Vythilingam, M. (2004). Deficits in verbal declarative memory function in women with childhood sexual abuse-related posttraumatic stress disorder. *Journal of Nervous and Mental Disease, 192*(10), 643-649. doi:10.1097/01.nmd.0000142027.52893.c8

Bryant, R. A., Moulds, M. L., Guthrie, R. M., Dang, S. T., & Nixon, R. D. (2003). Imaginal exposure alone and imaginal exposure with cognitive restructuring in treatment of posttraumatic stress disorder. *Journal of Consulting and Clinical Psychology, 71*(4), 706-712.

Carter, R. M., Hofstotter, C., Tsuchiya, N., & Koch, C. (2003). Working memory and fear conditioning. *Proceedings of the National Academy of Sciences of the United States of America, 100*(3), 1399-1404. doi:10.1073/pnas.0334049100

Charney, D. S. (2004). Psychobiological mechanisms of resilience and vulnerability: implications for successful adaptation to extreme stress. *American Journal of Psychiatry, 161*(2), 195-216. doi:10.1176/appi.ajp.161.2.195

Davis, M., & Whalen, P. J. (2001). The amygdala: vigilance and emotion. *Molecular Psychiatry, 6*(1), 13-34.

Ehlers, A., & Clark, D. M. (2000). A cognitive model of posttraumatic stress disorder. *Behaviour Research and Therapy, 38*, 319-345.

Ehlers, A., Mayou, R. A., & Bryant, B. (2003). Cognitive predictors of posttraumatic stress disorder in children: results of a prospective longitudinal study. *Behaviour Research and Therapy, 41*(1), 1-10.

Foa, E. B., Hembree, E. A., Cahill, S. P., Rauch, S. A., Riggs, D. S., Feeny, N. C., & Yadin, E. (2005). Randomized trial of prolonged exposure for posttraumatic stress disorder with and without cognitive restructuring: outcome at academic and community clinics. *Journal of Consulting and Clinical Psychology, 73*(5), 953-964. doi:10.1037/0022-006x.73.5.953

Fu, J., Li, P., Ouyang, X., Gu, C., Song, Z., Gao, J., . . . Hu, B. (2007). Rapid eye movement sleep deprivation selectively impairs recall of fear extinction in hippocampus-independent tasks in rats. *Neuroscience, 144*(4), 1186-1192. doi:10.1016/j.neuroscience.2006.10.050

Gazendam, F. J., & Kindt, M. (2012). Worrying affects associative fear learning: A startle fear conditioning study. *PLoS ONE, 7*(4). doi:10.1371/journal.pone.0034882

Germain, A. (2013). Sleep disturbances as the hallmark of PTSD: where are we now? *The American Journal of Psychiatry, 170*(4), 372-382. doi:10.1176/appi.ajp.2012.12040432

Gilbertson, M. W., Paulus, L. A., Williston, S. K., Gurvits, T. V., Lasko, N. B., Pitman, R. K., & Orr, S. P. (2006). Neurocognitive function in monozygotic twins discordant for combat exposure: Relationship to posttraumatic stress disorder. *Journal of Abnormal Psychology, 115*(3), 484-495. doi:10.1037/0021-843x.115-3.484

Glover, E. M., Jovanovic, T., Mercer, K. B., Kerley, K., Bradley, B., Ressler, K. J., & Norrholm, S. D. (2012). Estrogen levels are associated with extinction deficits in women with posttraumatic stress disorder. *Biological Psychiatry, 72*(1), 19-24. doi:10.1016/j.biopsych.2012.02.031

Glover, E. M., Mercer, K. B., Norrholm, S. D., Davis, M., Duncan, E., Bradley, B., . . . Jovanovic, T. (2013). Inhibition of fear is differentially associated with cycling estrogen levels in women. *Journal of Psychiatry & Neuroscience, 38*(5), 341-348. doi:10.1503/jpn.120129

Grady, A. K., Bowen, K. H., Hyde, A. T., Totsch, S. K., & Knight, D. C. (2016). Effect of continuous and partial reinforcement on the acquisition and extinction of human conditioned fear. *Behavioral Neuroscience, 130*(1), 36-43. doi:10.1037/bne0000121

Graham, B. M., & Milad, M. R. (2013). Blockade of estrogen by hormonal contraceptives impairs fear extinction in female rats and women. *Biological Psychiatry, 73*(4), 371-378. doi:10.1016/j.biopsych.2012.09.018

Grigorovich, A., Gomez, M., Leach, L., & Fish, J. (2013). Impact of posttraumatic stress disorder and depression on neuropsychological functioning in electrical injury survivors. *Journal of Burn Care & Research, 34*(6), 659-665. doi:10.1097/BCR.0b013e31827e5062

Guthrie, R. M., & Bryant, R. A. (2006). Extinction learning before trauma and subsequent posttraumatic stress. *Psychosomatic Medicine, 68*, 307-311. doi:10.1097/01.psy.0000208629.67653.cc

Hayes, A. F. (2013). *Introduction to mediation, moderation, and conditional process analysis: A regression-based approach*. New York: The Guilford Press.

Hermans, D., Craske, M. G., Mineka, S., & Lovibond, P. F. (2006). Extinction in human fear conditioning. *Biological Psychiatry, 60*(4), 361-368. doi:10.1016/j.biopsych.2005.10.006

Holmes, A., & Singewald, N. (2013). Individual differences in recovery from traumatic fear. *Trends in Neurosciences, 36*(1), 23-31. doi:10.1016/j.tins.2012.11.003

Inslicht, S. S., Metzler, T. J., Garcia, N. M., Pineles, S. L., Milad, M. R., Orr, S. P., . . . Neylan, T. C. (2013). Sex differences in fear conditioning in posttraumatic stress disorder. *Journal of Psychiatric Research, 47*, 64-71. doi:10.1016/j.jpsychires.2012.08.027

Joëls, M., & Baram, T. Z. (2009). The neuro-symphony of stress. *Nature Reviews Neuroscience, 10*(6), 459-466. doi:10.1038/nrn2632

Jovanovic, T., Kazama, A., Bachevalier, J., & Davis, M. (2012). Impaired safety signal learning may be a biomarker of PTSD. *Neuropharmacology, 62*(2), 695-704. doi:10.1016/j.neuropharm.2011.02.023

Jovanovic, T., Keyes, M., Fiallos, A., Myers, K. M., Davis, M., & Duncan, E. J. (2005). Fear potentiation and fear inhibition in a human fear-potentiated startle paradigm. *Biological Psychiatry, 57*(12), 1559-1564. doi:10.1016/j.biopsych.2005.02.025

Jovanovic, T., & Norrholm, S. D. (2011). Neural mechanisms of impaired fear inhibition in posttraumatic stress disorder. *Frontiers in Behavioral Neuroscience, 5*, 44. doi:10.3389/fnbeh.2011.00044

Jovanovic, T., Norrholm, S. D., Blanding, N. Q., Davis, M., Duncan, E., Bradley, B., & Ressler, K. J. (2010). Impaired fear inhibition is a biomarker of PTSD but not depression. *Depression and Anxiety, 27*(3), 244-251. doi:10.1002/da.20663

Jovanovic, T., Nylocks, K. M., Gamwell, K. L., Smith, A., Davis, T. A., Norrholm, S. D., & Bradley, B. (2014). Development of fear acquisition and extinction in children: effects of age and anxiety. *Neurobiology of Learning and Memory, 113*, 135-142. doi:10.1016/j.nlm.2013.10.016

Jovanovic, T., & Ressler, K. J. (2010). How the neurocircuitry and genetics of fear inhibition may inform our understanding of PTSD. *American Journal of Psychiatry, 167*(6), 648-662. doi:10.1176/appi.ajp.2009.09071074

Kalsbeek, A., van der Spek, R., Lei, J., Endert, E., Buijs, R. M., & Fliers, E. (2012). Circadian rhythms in the hypothalamo-pituitary-adrenal (HPA) axis. *Molecular and Cellular Endocrinology, 349*(1), 20-29. doi:10.1016/j.mce.2011.06.042

Kindt, M., & Soeter, M. (2013). Reconsolidation in a human fear conditioning study: a test of extinction as updating mechanism. *Biological Psychology, 92*(1), 43-50. doi:10.1016/j.biopsycho.2011.09.016

Li, S. S., & McNally, G. P. (2014). The conditions that promote fear learning: prediction error and Pavlovian fear conditioning. *Neurobiology of Learning and Memory, 108*, 14-21. doi:10.1016/j.nlm.2013.05.002

Liberzon, I., & Abelson, J. L. (2016). Context processing and the neurobiology of post-traumatic stress disorder. *Neuron, 92*(1), 14-30. doi:10.1016/j.neuron.2016.09.039

Lommen, M. J. J., Engelhard, I. M., Sijbrandij, M., van den Hout, M. A., & Hermans, D. (2013). Pre-trauma individual differences in extinction learning predict posttraumatic stress. *Behaviour Research and Therapy, 51*(2), 63-67. doi:10.1016/j.brat.2012.11.004

Lovibond, P. F. (2004). Cognitive processes in extinction. *Learning and Memory, 11*(5), 495-500. doi:10.1101/lm.79604

Lovibond, P. F., Davis, N. R., & O'Flaherty, A. S. (2000). Protection from extinction in human fear conditioning. *Behaviour Research and Therapy, 38*(10), 967-983.

Lovibond, S. H., & Lovibond, P. F. (1995). *Manual for the Depression Anxiety Stress Scales* (2nd ed.). Sydney: Psychology Foundation.

Marks, I., Lovell, K., Noshirvani, H., Livanou, M., & Thrasher, S. (1998). Treatment of posttraumatic stress disorder by exposure and/or cognitive restructuring: a controlled study. *Archives of General Psychiatry, 55*(4), 317-325.

McFarlane, A. C., Barton, C. A., Yehuda, R., & Wittert, G. (2011). Cortisol response to acute trauma and risk of posttraumatic stress disorder. *Psychoneuroendocrinology, 36*(5), 720-727. doi:10.1016/j.psyneuen.2010.10.007

Milad, M. R., Orr, S. P., Lasko, N. B., Chang, Y., Rauch, S. L., & Pitman, R. K. (2008). Presence and acquired origin of reduced recall for fear extinction in PTSD: Results of a twin study. *Journal of Psychiatric Research, 42*(7), 515-520. doi:10.1016/j.jpsychires.2008.01.017

Milad, M. R., Orr, S. P., Pitman, R. K., & Rauch, S. L. (2005). Context modulation of memory for fear extinction in humans. *Psychophysiology, 42*(4), 456-464. doi:10.1111/j.1469-8986.2005.00302.x

Milad, M. R., Pitman, R. K., Ellis, C. B., Gold, A. L., Shin, L. M., Lasko, N. B., . . . Rauch, S. L. (2009). Neurobiological basis of failure to recall extinction memory in posttraumatic stress disorder. *Biological Psychiatry, 66*(12), 1075-1082. doi:10.1016/j.biopsych.2009.06.026

Milad, M. R., Zeidan, M. A., Contero, A., Pitman, R. K., Klibanski, A., Rauch, S. L., & Goldstein, J. M. (2010). The influence of gonadal hormones on conditioned fear extinction in healthy humans. *Neuroscience, 168*(3), 652-658. doi:10.1016/j.neuroscience.2010.04.030

Mineka, S., & Oehlberg, K. (2008). The relevance of recent developments in classical conditioning to understanding the etiology and maintenance of anxiety disorders. *Acta Psychologica, 127*(3), 567-580. doi:10.1016/j.actpsy.2007.11.007

Mueller, D., & Cahill, S. P. (2010). Noradrenergic modulation of extinction learning and exposure therapy. *Behavioural Brain Research, 208*(1), 1-11. doi:10.1016/j.bbr.2009.11.025

Myers, K. M., & Davis, M. (2002). Behavioral and Neural Analysis of Extinction. *Neuron, 36*(4), 567-584. doi:10.1016/S0896-6273(02)01064-4

Myers, K. M., & Davis, M. (2007). Mechanisms of fear extinction. *Molecular Psychiatry, 12*(2), 120-150. doi:10.1038/sj.mp.4001939

National Center for Posttraumatic Stress Disorder. (n.d.). Using the PTSD Checklist for DSM-IV (PCL). Retrieved from http://www.ptsd.va.gov/professional/pages/assessments/assessment-pdf/PCL-handout.pdf

Norrholm, S. D., Jovanovic, T., Vervliet, B., Myers, K. M., Davis, M., Rothbaum, B. O., & Duncan, E. J. (2006). Conditioned fear extinction and reinstatement in a human fear-potentiated startle paradigm. *Learning & Memory, 13*(6), 681-685. doi:10.1101/lm.393906

Norrholm, S. D., Vervliet, B., Jovanovic, T., Boshoven, W., Myers, K. M., Davis, M., . . . Duncan, E. J. (2008). Timing of extinction relative to acquisition: a parametric analysis of fear extinction in humans. *Behavioral Neuroscience, 122*(5), 1016-1030. doi:10.1037/a0012604

Norrholm, S. D., Jovanovic, T., Olin, I. W., Sands, L. A., Karapanou, I., Bradley, B., & Ressler, K. J. (2011). Fear extinction in traumatized civilians with posttraumatic stress disorder: Relation to symptom severity. *Biological Psychiatry, 69*(6), 556-563. doi:10.1016/j.biopsych.2010.09.013

Orr, S. P., Lasko, N. B., Macklin, M. L., Pineles, S. L., Chang, Y., & Pitman, R. K. (2012). Predicting post-trauma stress symptoms from pre-trauma psychophysiologic reactivity, personality traits and measures of psychopathology. *Biology of Mood & Anxiety Disorders, 2*(8).

Orr, S. P., Metzger, L. J., Lasko, N. B., Macklin, M. L., Peri, T., & Pitman, R. K. (2000). De novo conditioning in trauma-exposed individuals with and without posttraumatic stress disorder. *Journal of Abnormal Psychology, 109*(2), 290-298. doi:10.1037/0021-843x.109.2.290

Orsini, C. A., & Maren, S. (2012). Neural and cellular mechanisms of fear and extinction memory formation. *Neuroscience and Biobehavioral Reviews, 36*(7), 1773-1802. doi:10.1016/j.neubiorev.2011.12.014

Pace-Schott, E. F., Germain, A., & Milad, M. R. (2015). Effects of sleep on memory for conditioned fear and fear extinction. *Psychological Bulletin*. doi:10.1037/bul0000014

Pace-Schott, E. F., Spencer, R. M. C., Vijayakumar, S., Ahmed, N. A. K., Verga, P. W., Orr, S. P., . . . Milad, M. R. (2013). Extinction of conditioned fear is better learned and recalled in the morning than in the evening. *Journal of Psychiatric Research, 47*(11), 1776-1784. doi:10.1016/j.jpsychires.2013.07.027

Pace-Schott, E. F., Tracy, L. E., Rubin, Z., Mollica, A. G., Ellenbogen, J. M., Bianchi, M. T., . . . Orr, S. P. (2014). Interactions of time of day and sleep with between-session habituation and extinction memory in young adult males. *Experimental Brain Research, 232*(5), 1443-1458. doi:10.1007/s00221-014-3829-9

Pace-Schott, E. F., Verga, P. W., Bennett, T. S., & Spencer, R. M. C. (2012). Sleep promotes consolidation and generalization of extinction learning in simulated exposure therapy for spider fear. *Journal of Psychiatric Research, 46*, 1036-1044. doi:10.1016/j.jpsychires.2012.04.015

Pineles, S. L., Nillni, Y. I., King, M. W., Patton, S. C., Bauer, M. R., Mostoufi, S. M., . . . Orr, S. P. (2016). Extinction retention and the menstrual cycle: Different associations for women with posttraumatic stress disorder. *Journal of Abnormal Psychology, 125*(3), 349-355. doi:10.1037/abn0000138

Pitman, R. K., Rasmusson, A. M., Koenen, K. C., Shin, L. M., Orr, S. P., Gilbertson, M. W., . . . Liberzon, I. (2012). Biological studies of post-traumatic stress disorder. *Nature, 13*, 769-787. doi:10.1038/nm3339

Porkka-Heiskanen, T., & Kalinchuk, A. V. (2011). Adenosine, energy metabolism and sleep homeostasis. *Sleep Medicine Reviews, 15*(2), 123-135. doi:10.1016/j.smrv.2010.06.005

Raes, A. K., De Raedt, R., Verschuere, B., & De Houwer, J. (2009). Failure to loose fear: The impact of cognitive load and trait anxiety on extinction. *Behaviour Research and Therapy, 47*(12), 1096-1101. doi:10.1016/j.brat.2009.08.002

Ross, R. J., Ball, W. A., Sullivan, K. A., & Caroff, S. N. (1989). Sleep disturbance as the hallmark of posttraumatic stress disorder. *American Journal of Psychiatry, 146*(6), 697-707.

Schelling, G., Kilger, E., Roozendaal, B., de Quervain, D. J., Briegel, J., Dagge, A., . . . Kapfhammer, H. P. (2004). Stress doses of hydrocortisone, traumatic memories, and symptoms of posttraumatic stress disorder in patients after cardiac surgery: a randomized study. *Biological Psychiatry, 55*(6), 627-633. doi:10.1016/j.biopsych.2003.09.014

Sevenster, D., Beckers, T., & Kindt, M. (2014). Fear conditioning of SCR but not the startle reflex requires conscious discrimination of threat and safety. *Frontiers in Behavioral Neuroscience, 8*, 32. doi:10.3389/fnbeh.2014.00032

Shvil, E., Sullivan, G. M., Schafer, S., Markowitz, J. C., Campeas, M., Wager, T. D., . . . Neria, Y. (2014). Sex differences in extinction recall in posttraumatic stress disorder: A pilot fMRI study. *Neurobiology of Learning and Memory*. doi:10.1016/j.nlm.2014.02.003

Silvestri, A. J., & Root, D. H. (2008). Effects of REM deprivation and an NMDA agonist on the extinction of conditioned fear. *Physiology & Behavior, 93*(1-2), 274-281. doi:10.1016/j.physbeh.2007.08.020

Soravia, L. M., Heinrichs, M., Aerni, A., Maroni, C., Schelling, G., Ehlert, U., . . . de Quervain, D. J. (2006). Glucocorticoids reduce phobic fear in humans. *Proceedings of the National Academy of Science of the United States of America, 103*(14), 5585-5590. doi:10.1073/pnas.0509184103

Soravia, L. M., Heinrichs, M., Winzeler, L., Fisler, M., Schmitt, W., Horn, H., . . . de Quervain, D. J. (2014). Glucocorticoids enhance in vivo exposure-based therapy of spider phobia. *Depression and Anxiety, 31*(5), 429-435. doi:10.1002/da.22219

Spoormaker, V. I., Schroter, M. S., Andrade, K. C., Dresler, M., Kiem, S. A., Goya-Maldonado, R., . . . Czisch, M. (2012). Effects of rapid eye movement sleep deprivation on fear extinction recall and prediction error signaling. *Human Brain Mapping, 33*, 2362-2376. doi:10.1002/hbm.21369

Spoormaker, V. I., Sturm, A., Andrade, K. C., Schroter, M. S., Goya-Maldonado, R., Holsboer, F., . . . Czisch, M. (2010). The neural correlates and temporal sequence of the relationship between shock exposure, disturbed sleep and impaired consolidation of fear extinction. *Journal of Psychiatric Research, 44*, 1121-1128. doi:10.1016/j.jpsychires.2010.04.017

Spring, J. D., Wood, N. E., Mueller-Pfeiffer, C., Milad, M. R., Pitman, R. K., & Orr, S. P. (2015). Prereactivation propranolol fails to reduce skin conductance reactivity to prepared fear-conditioned stimuli. *Psychophysiology, 52*(3), 407-415. doi:10.1111/psyp.12326

van Ast, V. A., Vervliet, B., & Kindt, M. (2012). Contextual control over expression of fear is affected by cortisol. *Frontiers in Behavioral Neuroscience, 6*, 67. doi:10.3389/fnbeh.2012.00067

Weathers, F. W., Litz, B. T., Huska, J. A., & Keane, T. M. (1994). *The PTSD Checklist-Civilian Version (PCL-C) for DSM-IV*. Boston: National Center for PTSD, Behavioral Sciences Division.

Wegerer, M., Kerschbaum, H., Blechert, J., & Wilhelm, F. H. (2014). Low levels of estradiol are associated with elevated conditioned responding during fear extinction and with intrusive memories in daily life. *Neurobiology of Learning and Memory, 116*, 145-154. doi:10.1016/j.nlm.2014.10.001

Weike, A. I., Schupp, H. T., & Hamm, A. O. (2007). Fear acquisition requires awareness in trace but not delay conditioning. *Psychophysiology, 44*(1), 170-180. doi:10.1111/j.1469-8986.2006.00469.x

Yehuda, R., Bierer, L. M., Pratchett, L. C., Lehrner, A., Koch, E. C., Van Manen, J. A., . . . Hildebrandt, T. (2015). Cortisol augmentation of a psychological treatment for warfighters with posttraumatic stress disorder: Randomized trial showing improved treatment retention and outcome. *Psychoneuroendocrinology, 51*, 589-597. doi:10.1016/j.psyneuen.2014.08.004

Zeidan, M. A., Igoe, S. A., Linnman, C., Vitalo, A., Levine, J. B., Klibanski, A., . . . Milad, M. R. (2011). Estradiol modulates medial prefrontal cortex and amygdala activity during fear extinction in women and female rats. *Biological Psychiatry, 70*(10), 920-927. doi:10.1016/j.biopsych.2011.05.016

Zoladz, P. R., & Diamond, D. M. (2013). Current status on behavioral and biological markers of PTSD: A search for clarity in a conflicting literature. *Neuroscience and Biobehavioral Reviews, 37*(5), 860-895. doi:10.1016/j.neubiorev.2013.03.024

Zuj, D. V., Palmer, M. A., Hsu, C. K., Nicholson, E. L., Cushing, P. J., Gray, K. E., & Felmingham, K. L. (2016). Impaired fear extinction associated with PTSD increases with hours-since-waking. *Depression and Anxiety, 33*(3), 203-210. doi:10.1002/da.22463

Zuj, D. V., Palmer, M. A., Lommen, M. J. J., & Felmingham, K. L. (2016). The centrality of fear extinction in linking risk factors to PTSD: A narrative review. *Neuroscience & Biobehavioral Reviews, 69*, 15-35. doi:10.1016/j.neubiorev.2016.07.014

Appendix A

Information Sheet and Consent Form

INFORMATION SHEET AND CONSENT FORM

Study title: Identification of Cognitive and Genetic Predictors of Posttraumatic Stress Disorder

Chief Investigator:

Prof Kim Felmingham **Department: School of Psychology, University of Tasmania**

Associate Investigators:

Mr Daniel Zuj **Department: School of Psychology, UTAS**

Dr Matt Palmer **Department: School of Psychology, UTAS**

Professor James Vickers **Department: School of Psychology, UTAS**

Before you decide whether or not you wish to participate in this study, it is important for you to understand why the research is being done and what it will involve. Please take the time to read the following information carefully and discuss it with the researchers if you wish.

What is the purpose of this study?

You are invited to participate in a research study that will investigate predictors of Posttraumatic Stress Disorder (PTSD) symptoms following exposure to trauma. Recent research suggests that impairments in fear extinction may be involved in PTSD, and genetic, cognitive and hormonal influences on fear extinction have been found. No studies have examined the effects of genetic, cognitive, hormonal influences on fear extinction prior to being exposed to traumatic events, and subsequent PTSD symptoms.

Why have I been invited to enter the study?

You have been invited to participate in this study because you are enrolled in the Bachelor of Paramedic Medicine and will be exposed to traumatic events (ambulance work) as part of your practicum experience.

What does the study involve?

If you agree to participate in this study, you will be screened for eligibility (you will need to be aged 18-40, be of Caucasian background, and have no psychiatric or neurological history) and asked to sign the Participant Consent Form. You will then come in for an assessment (which will take approximately 90 minutes) at the Cognitive Neuroscience Laboratory in the School of Psychology, UTAS prior to your first practicum placement. In this assessment, you will complete the following components listed below. Three and six months after your practicum placement, you will receive a follow-up phone call (each approximately 30 minutes) by an intern clinical psychologist to assess your level of PTSD symptoms, your mood and any current stressors and social support.

Genetic Analysis

You will be asked to provide a saliva sample for genetic (DNA) analysis by placing saliva into a small plastic tube. This sample will be de-identified and given an ID number, it will be analysed for the BDNF, 5hTTLPR, COMT, PACAP and FKBP5 genotypes (which are all associated with fear processing and extinction) at the pathology labs in the School of Medicine, UTAS. Following genotyping, the saliva sample will be immediately destroyed. You will not be provided with individual information from this genetic analysis as these genotypes are not associated with any known disease states.

Hormonal Analysis

You will be asked to provide a saliva sample for hormonal analysis by placing saliva into a small plastic tube. This sample will be de-identified and given an ID number, it will be analysed for the stress hormones (noradrenaline and cortisol) and estrogen (which are associated with fear extinction) at the pathology labs in the School of Medicine, UTAS. Following processing, the saliva sample will be immediately destroyed.

Clinical and Neuropsychological Assessment

You will be administered a number of questionnaires which will assess your mood, your previous trauma experience, and your psychiatric history. You will complete two cognitive assessments examining verbal and memory function. These questionnaires and tests will take approximately 20 minutes.

Fear Conditioning and Extinction Task.

You will then complete a standard fear conditioning and extinction task. This task involves viewing different coloured circles on a screen (red, or blue), on presentation of some of these circles, you will be administered a mild electric shock. You will set the level of shock before getting

commencing this task as feeling "uncomfortable but not painful." Your body arousal will be measured by recording your skin conductance from two small electrodes placed on your fingers. This is a widely used and standardized procedure to examine fear extinction.

Follow-up Telephone Clinical Assessment

Three and six months following your practicum placement, you will receive a 30 minute phone call from an intern clinical psychologist. This phone call is designed to re-assess your PTSD symptoms, your mood, your current stressful life events and level of social support.

Are there any risks?

Some participants may feel a mild level of discomfort or anxiety in receiving the mild electrical shock as part of the fear conditioning and extinction task. However, you can set the level of this shock that you feel comfortable with before commencing the study, so it is expected that you will feel minimal discomfort or arousal. Your possible discomfort and distress will be monitored by the researcher (Daniel Zuj) who will be present throughout the task. You may stop the study and withdraw from the study at any time without penalty. Withdrawing from the study will not affect your treatment or potential treatment at the Centre for Traumatic Stress Studies in any way.

If you do feel upset or uncomfortable at any point please let the researcher conducting your assessment know.

Some individuals may feel slightly embarrassed by providing a saliva sample. You can provide this sample behind a screen or in the bathroom if you request it.

Are there any benefits?

We cannot guarantee or promise that you will receive any benefits from this study. You may learn more about psychological research, you will have your psychological reactions to your practicum placement more closely monitored and you may gain further knowledge about PTSD after receiving group results from the study.

What happens if I don't want to take part in the study?

Participation in this study is voluntary. It is completely up to you whether or not you participate. If you decide not to participate, it will not affect your current or future studies, or your relationship with the University of Tasmania. If you wish to withdraw from the study once it has started, you can do so at any time without having to give a reason and without penalty.

How will my confidentiality be protected?

All aspects of this study, including results will be strictly confidential and only the researchers will have access to your personal information. Any publication of results will only use de-identified information. Confidentiality will be maintained at all times and information will not be made available to participants or others outside the study. Original data will be stored in a locked office, and entered into a database that will be password protected. Electronic data will de-identified using a unique ID number and stored on protected hard-drives within locked offices. Data is not analysed individually, but only as part of larger group comparative analyses.

What happens with the results?

If you give us your permission by signing the consent document, we plan to discuss/publish the results amongst the researchers on this project, the HREC for monitoring purposes, the funding body for monitoring purposes, peer-review journals, presentation at conferences or other professional forums. Results will also be discussed at community meetings and in consultation with refugee advisory groups we are collaborating with. You will be invited to participate in these discussions.

You will receive information about your psychological functioning at the three and six month follow-up assessments.

Will taking part in this study cost me anything, and will I be paid?

Participation in this study will not cost you anything. In addition, you will be reimbursed $50 for completing the study to cover your expenses.

How is this study being paid for?

This study is being sponsored by the National Health and Medical Research Council (NHMRC). No commercial bodies have an interest in this research project.

Who should I contact if I have concerns about the conduct of this study?

This study has been approved by the Tasmanian Health and Medical Human Research Ethics Committee. Any person with concerns or complaints about the conduct of this study should contact the Research Office who is nominated to receive complaints from research participants. You should contact them on 02 9926 8106 and quote [HREC project No H0013412]

Thank you for taking the time to consider this study.

If you wish to take part in it, please sign the attached consent form.

This information sheet is for you to keep.

CONSENT FORM

Study title: Identification of Cognitive and Genetic Predictors of Posttraumatic Stress Disorder

1. I,.....
   of.....
   agree to participate as a subject in the study described in the participant information statement set out above.
2. I acknowledge that I have read the participant information statement, which explains why I have been selected, the aims of the study and the nature and the possible risks of the investigation, and the statement has been explained to me to my satisfaction.
3. Before signing this consent form, I have been given the opportunity of asking any questions relating to any possible physical and mental harm I might suffer as a result of my participation and I have received satisfactory answers.
4. I understand that I can withdraw from the study at any time without prejudice to my relationship to University of Tasmania
5. I agree that research data gathered from the results of the study may be published, provided that I cannot be identified.
6. I understand that if I have any questions relating to my participation in this research, I may contact Professor Kim Felmingham or Mr Daniel Zuj on telephone - 62261965 who will be happy to answer them. You may also email: Kim.Felmingham@utas.edu.au and a researcher will be in contact with you.
7. I acknowledge receipt of a copy of this Consent Form and the Participant Information Statement.

Complaints may be directed to the Research Office, University of Tasmania, 62262764.

Signature of subject	**Please PRINT name**	**Date**
Signature of investigator	**Please PRINT name**	**Date**
Signature of interpreter	**Please PRINT name**	**Date**